

**İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY**

**CARBON REMOVAL AND ELECTRICITY GENERATION BY USING  
STARCH AS SUBSTRATE**

**M.Sc. Thesis by  
Özlem ARSLAN**

**Department : Environmental Engineering**

**Programme : Environmental Biotechnology**

**JANUARY 2011**







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**JANUARY 2011**



**İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ**

**MİKROBİYAL YAKIT HÜCRESİNDE NİŞASTANIN SÜBSTRAT OLARAK  
KULLANILDIĞI ŞARTLARDA KARBON GİDERİMİ VE ELEKTRİK ÜRETİMİ**

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## **FOREWORD**

I am deeply indebted to my thesis supervisor, Assoc. Prof. Dr. Ozlem KARAHAN, who helped me throughout my study with advice, guidance and encouragement.

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## ABBREVIATIONS

<b>ASP</b>	: Activated Sludge Process
<b>BES</b>	: Bioelectrochemical Systems
<b>BOD</b>	: Biochemical Oxygen Demand
<b>CE</b>	: Coloumbic Efficiency
<b>CEM</b>	: Cation Exchange Membrane
<b>COD</b>	: Chemical Oxygen Demand
<b>DO</b>	: Dissolved Oxygen
<b>I</b>	: Current
<b>I<sub>AN</sub></b>	: Current Density (Anode)
<b>KOİ</b>	: Kimyasal Oksijen İhtiyacı
<b>MFC</b>	: Microbial Fuel Cell
<b>MLSS</b>	: Mixed Liquor Suspended Solids
<b>MLVSS</b>	: Mixed Liquor Volatile Suspended Solids
<b>OCV</b>	: Open Circuit Voltage
<b>P</b>	: Power
<b>P<sub>AN</sub></b>	: Power Density (Anode)
<b>P</b>	: Power
<b>PEM</b>	: Proton Exchange Membrane
<b>SPW</b>	: Starch Processing Wastewater
<b>TEA</b>	: Terminal Electron Acceptor
<b>UAKM</b>	: Uçucu Askıda Katı Madde



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## **CARBON REMOVAL AND ELECTRICITY GENERATION BY USING STARCH AS SUBSTRATE**

### **SUMMARY**

Microbial fuel cell (MFC) is an emerging technology that converts the energy contained in organic matter directly to useful electrical power. In recent years, research activity on microbial fuel cell (MFC) technology has increased markedly. Microbial fuel cell (MFC) is a sustainable technology in recent years because of their capability to simultaneously generate electricity and treat organic wastewaters. The working principle of a MFC is based on the catalytic activity of microorganisms to oxidize organic substrate in an anaerobic anode chamber to generate electrons and protons.

In this work, the production of electricity and the oxidation of the pollutants contained in a synthetic wastewater fed with starch as the carbon sources, using a mediator-less two-compartment microbial fuel cell (MFC) has been studied. This thesis consists of three stages.

At the beginning the activated sludge which was taken from Bahçeşehir Domestic Wastewater Treatment Plant has been acclimated to starch under laboratory conditions for 1.5 months.

Secondly, MFC start-up phase has been carried out.

Last phase consisted of experiments in MFC. During the MFC experiment phase, special attention has been paid in which it was found that with high hydraulic and solid retention times it is possible to obtain a very efficient process with a Chemical Oxygen Demand (COD) removal and electricity generation. MFC operation with sludge concentration has been studied, with the system having a volatile suspended solids concentration (MLVSS) of, 1500 mg/l. Moreover, synthetic wastewater with the different COD concentrations, have been studied.



# **MİKROBİYAL YAKIT HÜCRESİNDE NİŞASTANIN SÜBSTRAT OLARAK KULLANILDIĞI ŞARTLARDA KARBON GİDERİMİ VE ELEKTRİK ÜRETİMİ**

## **ÖZET**

Mikrobiyal yakıt hücreleri (MYH) enerji içeren organik maddeyi doğrudan elektriksel güce çeviren, gelişmekte olan bir teknolojidir. Son yıllarda, mikrobiyal yakıt hücresi (MYH) teknolojisi üzerindeki araştırma faaliyeti belirgin olarak artmıştır. Mikrobiyal yakıt hücreleri (MYH) eş zamanlı elektrik üretimi ve organik madde giderimi dolayısıyla son yıllarda sürdürülebilir bir teknoloji olarak yerini almıştır. MYH çalışma prensibi mikroorganizmaların elektron ve proton üretmek için anaerobik anot hücresinde organik maddeleri okside etmek için kullandıkları katabolik aktiviteye dayanır.

Bu çalışmada, elektrik üretimi ve iki bölmeli mikrobiyal yakıt hücresi (MYH) kullanılarak karbon kaynağı olarak nişasta ile beslenen bir sentetik atıksu içindeki kirletici maddelerin oksidasyonu incelenmiştir. Bu kapsamda, bu tez üç aşamadan oluşmaktadır.

Bahçeşehir Evsel Atıksu Arıtma Tesisinden alınan aktif çamur 1.5 ay boyunca laboratuvarında nişastaya aklime edilmiştir.

İkincisi, MYH kurulum aşaması yapılmıştır.

Son aşama MYH ile gerçekleştirilen deneylerinden oluşmuştur. MYH deneyleri aşamasında, özel olarak yüksek hidrolik bekletme süreleri ve çamur yaşları ile Kimyasal Oksijen İhtiyacı (KOİ) giderimi ve elektrik üretiminde çok verimli sonuçlar elde etmenin mümkün olduğu tespit edilmiştir. MYH işletmesinde yüksek çamur konsantrasyonu incelenmiş ve sistem uçucu askıda katı madde konsantrasyonu(UAKM); 1500 mg/L ile işletilmiştir. Sistemde aynı kompozisyonda sentetik atıksu, ancak farklı KOİ konsantrasyonlarının beslenmesi durumu incelenmiştir.



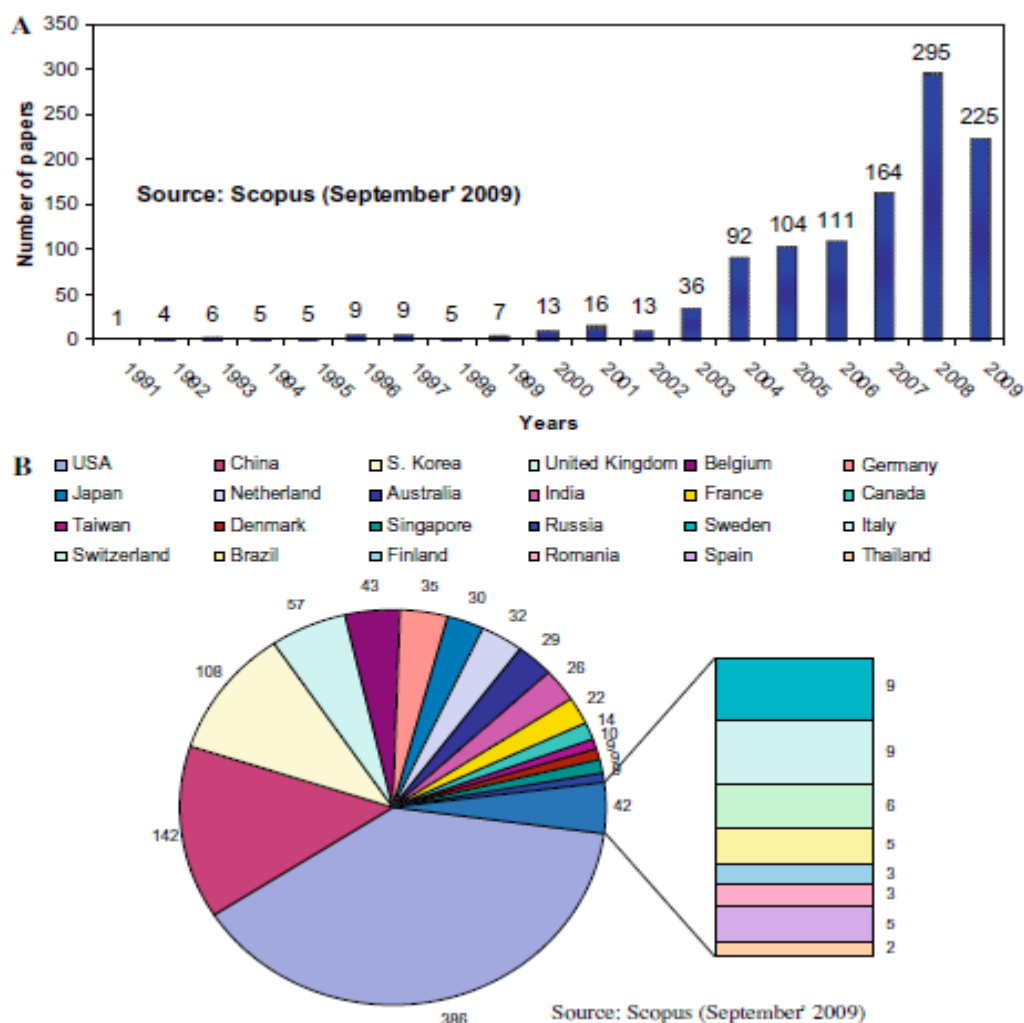
## **1. INTRODUCTION**

### **1.1. Meaning and Significance of the Thesis**

Microbial fuel cells (MFCs) have emerged in recent years as a promising yet challenging technology. Microbial fuel cells (MFCs) have gained a lot of attention as a mode of converting organic waste including low-strength wastewaters and lignocellulosic biomass into electricity.

In an MFC, microorganisms interact with electrodes using electrons, which are either removed or supplied through an electrical circuit (Rabaey et al., 2007). MFCs are the major type of bioelectrochemical systems (BESs) which convert biomass spontaneously into electricity through the metabolic activity of the microorganisms. MFC is considered to be a promising sustainable technology to meet increasing energy needs, especially using wastewaters as substrates, which can generate electricity and accomplish wastewater treatment simultaneously, thus may lower the operational costs of wastewater treatment plant (Lu et al., 2009).

The real interest in MFCs has tremendously grown in recent years, both in terms of number of researchers as well as the applications for these systems. Fig. 1.1A shows that ‘Scopus’ search with keyword “microbial fuel cell” which almost 60-fold increase in the number of articles published over the last decade (1998–2008). Moreover, the reported electric current output from the MFCs has also increased tremendously over the recent years. Fig. 1.1 B shows the country-wise distribution of MFC researchers, the data for which was also drawn from ‘Scopus’. It is evident that the interest in MFC research is truly global with more and more researchers coming up from different countries.



**Figure 1.1:** (A) The number of articles on MFCs. The data is based on the number of articles mentioning MFC in the citation database Scopus in September 2009. (B) The country-wise distribution in MFC research. The data is based on the number of articles mentioning MFC in the citation database Scopus in September 2009.

## 1.2. Purpose and Scope of the Thesis

The aim of thesis is to study the performance of an MFC fed with synthetic wastewater consisting of starch as a substrate. The work is focussed on the study of acclimation of the microbial culture to starch and one effect of the biodegradability of the substrate, paying special attention to the study of the relationship between COD removal and electricity production, including the achievement of a high power and current density. The hydraulic and solid retention times of the MFC were high enough to assure the degradation of the organic substrate. A two-compartment MFC with the anodic and the cathodic chambers separated by a proton exchange membrane is used. Carbon removal



and electricity generation efficiencies have been observed for different starch concentrations which were 525 mg COD/l, 1050 mg COD/l and 250 mg COD/l.

The first chapter of the Thesis, covers the meaning and importance of the subject and, the purpose and scope of the Thesis.

In the second chapter, a review on MFC with emphases on the recent advances in MFC reactor designs, MFC performances, applications and optimization of important operating parameters and a brief MFC history has been presented.

In the third chapter, methods used in experimental studies, materials used and the applied analysis methods have been given.

In the fourth chapter, experimental studies are presented. The data obtained from experimental studies are shown and interpreted.

In the fifth chapter, a general evaluation of the experimental studies and results are presented.



## **2. LITERATURE SURVEY**

### **2.1. Generating Electricity from MFC**

Microbial fuel cell (MFC ) technologies represent the newest approach for generating electricity – bioelectricity generation from biomass using bacteria. While the first observation of electrical current generated by bacteria is generally created to Potter in 1911 ( Potter 1911), very few practical advances were achieved in this field even 55 years later (Lewis 1966). In the early 1990s, fuel cells became of more interest and work on MFCs began to increase (Allen and Bennetto 1993). However, experiments that were conducted required the use of chemical mediators, or electron shuttles, which could carry electrons from inside the cell to exogenous electrodes. The breakthrough in MFCs occurred in 1999 when it was recognized that mediators did not need to be added (Kim et al. 1999c; Kim et al. 1999d).

### **2.2. Principles of MFC**

In an MFC, microorganisms degrade organic matter, producing electrons that travel through a series of respiratory enzymes in the cell and make energy for the cell in the form of ATP. The electrons are then released to a terminal electron acceptor (TEA) which accepts the electrons and becomes reduced. For example, oxygen can be reduced to water through a catalyzed reaction of the electrons with protons. Many TEAs such as oxygen, nitrate, sulfate, and others readily diffuse into the cell where they accept electrons forming products can diffuse out of the cell. However, we now know that some bacteria can transfer electrons exogeneously to a TEA such as a metal oxide like iron oxide. It is these bacteria that can exogenously transfer electrons, called exoelectrogens, that can be used to produce power in an MFC. The nomenclature used for categorizing process, microorganisms, and reactors for methane generation is: methanogenesis, methanogens, and anaerobic digesters. Similarly, we classify this method of electron-generating process as electrogenesis, with the bacteria exoelectrogens and the reactor a microbial fuel cell (MFC).

Bacterial reactions can be carried out over several different temperature ranges depending on the tolerance of the bacteria, ranging from moderate or room-level temperatures (15-35<sup>0</sup>C) to both high temperatures (50-60<sup>0</sup>C) tolerated by thermophiles and low temperatures (< 15<sup>0</sup>C) where psychrophiles can grow. Virtually any biodegradable organic matter can be used in an MFC, including volatile acids, carbohydrates, proteins, alcohols, and even relatively recalcitrant materials like cellulose.

### **2.3. Advantages and Challenges of MFCs**

Production of a useful product in the form of electricity: The current generated is dependent on the wastewater strength and the Coulombic efficiency.

Lack of a need for aeration: No aeration is needed for an air-cathode MFC that uses only passive oxygen transfer at the cathode.

Reduced solids production: The MFC is an anaerobic process, and thus bacterial biomass production will be reduced compared to that of an aerobic system. Solids treatment is expensive, and using an MFC may substantially reduce solids production.

Despite the fact that in recent years the power generation from MFCs have improved considerably and also reached the level of primary power target at least in small lab-scale systems, the scale-up is still a big challenge. Moreover, the high cost of cation exchange membranes, the potential for biofouling and associated high internal resistance restrain the power generation and limit the practical application of MFCs (Hu, 2008). In case of phototrophic MFCs, the need for artificial illumination (Strik et al., 2008b; He et al., 2009) exerts extra energy input for the system and raises the cost. Domestic wastewater, which has organic matter with embedded energy content, contains almost 10 times the energy needed to treat it (WERF, 2009). While emerging technologies are promising, none of the processes available today can yet fully extract all the energy available in wastewater without further investment in their research and development. The actual performance of BESs with reactor volumes larger than 1 L is still lower than the goal of 1 kW/m<sup>3</sup>, which is considered as the threshold for feasible industrial application for energy recovery from organic matter (Pham et al., 2009b). A major drawback associated with MFCs is the start-up time which may vary from 4 to 103 days depending on the inoculum, electrode materials, reactor design, operating conditions (temperature, external loading, etc.) but most

importantly on the substrate being fed into the system (Wang et al., 2009a). Another significant impediment in scaling up of MFCs for wastewater treatment is the lack of buffer capacity of electrolytes (You et al., 2009). There are several ways by which the existing limitations in MFCs could be overcome. It is agreed that the power output of most MFCs is too low for any envisioned applications (Lovley, 2008). Besides, the high cost of a precious metal catalyst such as platinum which is usually needed on a cathode is also a big hindrance in up-scaling of these systems. Open air biocathodes proposed by Clauwaert et al. (2007b) could be a possible solution in future. The replacement of platinized cathodes with non-platinized ones with a similar efficiency is a major improvement in this area (Van Bogaert et al., 2009; Zhang et al., 2009a). The use of manganese dioxide as an alternative cathode catalyst in MFCs (Zhang et al., 2009b) and stainless steel and nickel alloys in MECs (Selembo et al., 2009) has also been suggested. Besides the substrates mentioned in this article, there are several other possible substrates which can be tried in these systems. For example, the wastewater from canning of fruits and vegetables (with a COD ranging between 1000 and 10,000 mg/L) the major waste product from dairy industry (with COD from 60,000 to 80,000 mg/L) waste and wastewater from live stock industry particularly slaughterhouses are all potential substrates for MFC. The effluent from cane-molasses based distilleries, which is highly rich in organic load and produced in enormous volumes (Pant and Adholeya, 2007) could also be a potential substrate for MFCs. Lovley (2009) suggested that despite the present slow rate of substrate conversion to electricity in BES, there are several other potential applications for microbe-electrode technology. This may be in the form of implanted medical devices using blood sugar as fuel, microbial transistors, circuits and electronic computing devices. Reactions at the bioanode can be directed towards the production of valuable compounds from inexpensive substrates (Pham et al., 2009b). The organic matter in waste streams can be used as the substrate for anodic microorganisms for the production of polyhydroxybutyrate (PHB) (Freguia et al., 2007). Carbon dioxide capture and conversion to useful compounds in a MFC is another lucrative application, that has partly been realized recently (Cao et al., 2009). These authors reported the possibility of direct electron transfer between a cathode and microorganisms for fixation of CO<sub>2</sub> in biomass. MFCs have also been used for treatment of recalcitrant compounds at the bioanode or cathode side. When contaminants serve as electron acceptors in the MFC cathode chamber, the

environmental benefits of MFCs could be greatly enhanced. Denitrifying MFCs in which microorganisms in the cathode performed a complete denitrification by using electrons supplied by microorganisms oxidizing acetate or glucose in the anode have been reported (Clauwaert et al., 2007a; Jia et al., 2008). Another manifestation of these systems is for the production of certain chemical compounds. Recently, the  $\text{H}_2\text{O}_2$  production at a carbon felt cathode using the electricity generated by a MFC was demonstrated by Zhu and Ni (2009). In the same system, p-nitrophenol was also degraded completely with a maximum power output of  $143 \text{ mW/ m}^2$ . Yet another application of these systems could be as biosensors for wastewaters (Kim et al., 2003). Di Lorenzo et al. (2009) reported a biochemical oxygen demand (BOD) biosensor based on singlechamber MFC with air cathode and running on artificial wastewater and obtained a good correlation between COD concentration and current output.

#### **2.4. History of MFC**

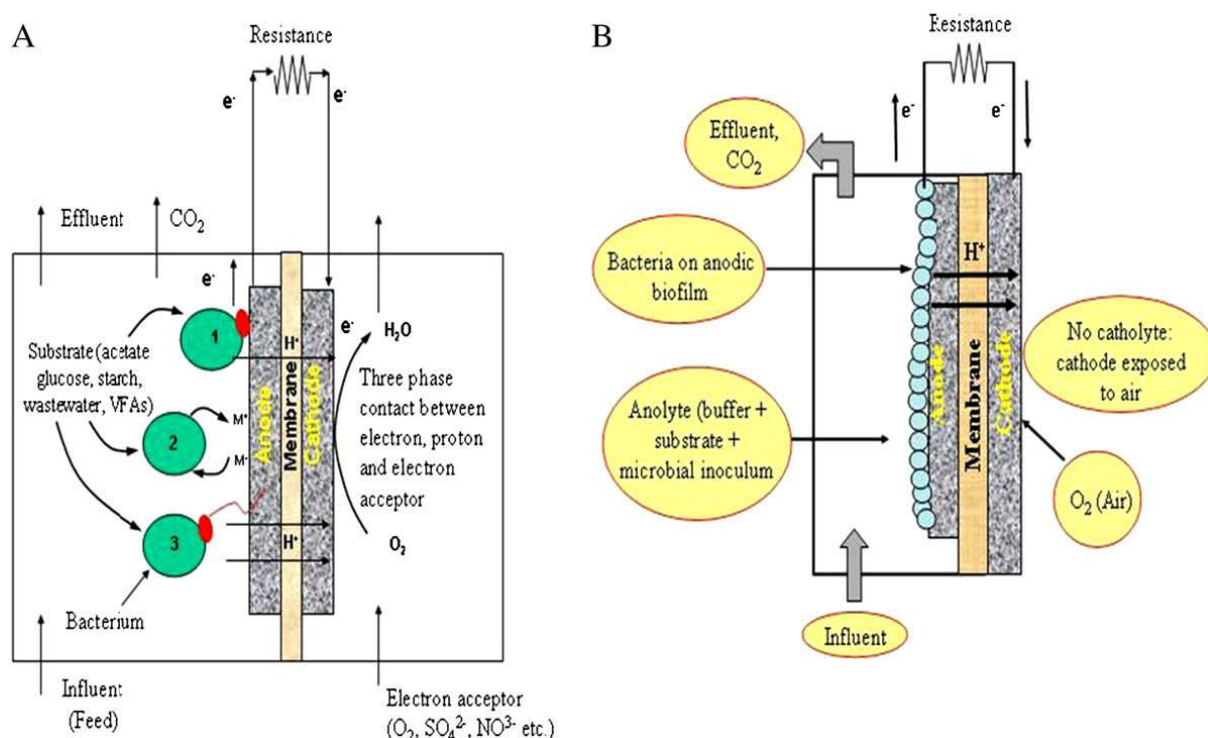
The knowledge that bacteria can generate electric current was first reported by Potter (1911). However, the real interest in MFCs has tremendously grown in recent years, both in terms of number of researchers as well as the applications for these systems as mentioned above. Moreover, the reported electric current output from the MFCs has also increased tremendously over the recent years. It is evident that the interest in MFC research is truly global with more and more researchers coming up from different countries. Over the past years, MFCs as a new source of bioenergy have been extensively reviewed. These include information on the various terminology and measurements used in these systems (Logan et al., 2006), state of the art information on MFCs and recent improvements in MFC technologies (Du et al., 2007), comparison of MFCs with conventional anaerobic digestion (Pham et al., 2006), practical implementation of BESs (Rozendal et al., 2008), bioanode performance in BES (Pham et al., 2009b), cathodic limitations in MFCs (Rismani-Yazdi et al., 2008). The mechanism of external electron transfer from two main bacteria in BES studies, *Geobacter sulfurreducens* and *Shewanella oneidensis* was described in great detail by Debabov (2008). ‘Microbial fuel cells’ (Logan, 2008) is another source of comprehensive information on MFCs. Logan (2009) presented the power densities for MFCs, normalized to electrode- projected surface areas reported over the years 1998–2008. However, a comprehensive review on the various substrates which have been used and can possibly be used in MFCs is still lacking.

Substrate is important for any biological process as it serves as carbon (nutrient) and energy source. The efficiency and economic viability of converting organic wastes to bioenergy depend on the characteristics and components of the waste material. Especially the chemical composition and the concentrations of the components that can be converted into products or fuels, is of major interest while considering the potential substrates in BES systems (Angenent and Wrenn, 2008). The substrate influences not only the integral composition of the bacterial community in the anode biofilm, but also the MFC performance including the power density (PD) and Coulombic Efficiency (CE) (Chae et al., 2009).

## **2.5. Design and Operation of MFCs**

An appropriate design is an important feature in MFCs and researchers have come up with several designs of MFCs over the years with improved performance (Du et al., 2007). Figure 2.1 (A) and (B) shows in detail the mode of operation and components of a typical two-chamber and a single-chamber MFC. In MFC, microorganisms oxidize organic matter in the anode chamber (anaerobic conditions) producing electrons and protons. Electrons transfer via the external circuit to the cathode chamber where electrons, protons and electron acceptor (mainly oxygen) combine to produce water (Li et al., 2009).

In a two-chamber set up, the anode and cathode compartments are separated by an ion-selective membrane, allowing proton transfer from anode to cathode and preventing oxygen diffusion to the anode chamber. In the single-chamber MFC, the cathode is exposed directly to the air. Besides these two common designs, several adaptations have been made in MFC design and structure.



**Figure 2.1:** (A) Simplified view of a two-chamber MFC with possible modes of electron transfer is shown. (1) Direct electron transfer (via outer membrane cytochromes); (2) electron transfer through mediators; and (3) electron transfer through nanowires. (B) Single – chamber MFC with open air cathode.

## 2.6. MFC Technologies for Wastewater Treatment

Microbial fuel cell (MFC) technologies are a promising and yet completely different approach to wastewater treatment as the treatment process can become a method of capturing energy in the form of electricity or hydrogen gas, rather than a drain on electrical energy. In the late 1990s, Kim and coworkers demonstrated that bacteria could be used in a biofuel cell as a method of determining the concentration of lactate in water (Kim *et al.* 1999d), and then that electricity generation in an MFC could be sustained by starch using an industrial wastewater (Kim *et al.* 1999). However, the power production was low and it was not clear whether the technology would have much impact on reducing wastewater strength. In 2004, this changed and the link between electricity using MFCs and wastewater treatment was clearly forged when it was demonstrated that domestic wastewater could be treated to practical levels while simultaneously generating electricity (Liu *et al.* 2004). The amount of electricity generated in this study, while low (26 mW/m<sup>2</sup>), was considerably higher (several orders of magnitude) than had previously been obtained using wastewater. Research led by Reimers (2001) a few years earlier had



demonstrated that organic and inorganic matter in marine sediments could be used in a novel type of MFC, making it apparent that a wide variety of substrates, materials, and system architectures could be used to capture electricity from organic matter with bacteria. Still, power levels in all these systems were relatively low. The final development that sparked the current interest in MFCs was provided by Rabaey *et al.* (2003) when they demonstrated power densities two orders of magnitude greater was possible in an MFC using glucose, again without the need for exogenous chemical mediators.

Following these demonstrations, the race was on to develop practical applications of MFCs, with the first goal being development of a scaleable technology for the treatment of domestic, industrial, and other types of wastewaters (Logan *et al.* 2006). While the energy that could be captured from wastewater is not enough to power a city, it is large enough to run a treatment plant. With advances, capturing this power could achieve energy sustainability of the water infrastructure. As an example of the power that can be derived from wastewater, consider the example that follows for energy recovery for a modest-sized town.

## **2.7. Substrates Used in MFCs**

In MFCs, substrate is regarded as one of the most important biological factors affecting electricity generation (Liu *et al.*, 2009). A great variety of substrates can be used in MFCs for electricity production ranging from pure compounds to complex mixtures of organic matter present in wastewater. So far the only objective of the various treatment processes is to remove pollutants from waste streams before their safe discharge to the environment. In the last century, activated sludge process (ASP) has been the mainstay of wastewater treatment. However, it is a very energy intensive process and according to an estimate, the amount of electricity needed to provide oxygen in ASPs in USA is equivalent to almost 2% of the total US electricity consumption (Electric Power Research Institute, 2002). At the same time, the addition of a second treatment step changes the status of several streams generated in the ASP treatment of agro-industry from “waste” to “raw material” which can eventually be utilized for the production of specific chemicals or energy (Kleerebezem and van Loosdrecht, 2007). Moreover, the emphasis of today’s waste management is on reuse and recovery of energy, which has led to new views on how these streams can be dealt with. Further, different researchers use different units to denote the performance of a MFC. One of the most common unit is current density,

which is either represented as the current generated per unit area of the anode surface area ( $\text{mA}/\text{cm}^2$ ) or current generated per unit volume of the cell ( $\text{mA}/\text{m}^3$ ).

### **2.7.1. Acetate**

In most of the MFC studies so far, acetate has been the substrate of choice for electricity generation. The recalcitrance of many types of wastewater makes them more difficult to be utilized as compared to acetate (Sun et al., 2009b). Acetate is a simple substrate and it is extensively used as carbon source to induce electroactive bacteria (Bond et al., 2002). In order to benchmark new MFC components, reactor designs or operational conditions, acetate is commonly used as a substrate because of its inertness towards alternative microbial conversions (fermentations and methanogenesis) at room temperature (Aelterman, 2009). Further, acetate is the end product of several metabolic pathways for higher order carbon sources (including the Entner–Doudoroff pathway for glucose metabolism) (Biffinger et al., 2008). Using a single-chambered MFC, Liu et al. (2005) reported that the power generated with acetate ( $506 \text{ mW}/\text{m}^2$ ,  $800 \text{ mg}/\text{L}$ ) was up to 66% higher than that produced with butyrate ( $305 \text{ mW}/\text{m}^2$ ,  $1000 \text{ mg}/\text{L}$ ). Very recently, Chae et al. (2009) compared the performance of four different substrates in terms of CE and power output. Acetate-fed MFC showed the highest CE (72.3%), followed by butyrate (43.0%), propionate (36.0%) and glucose (15.0%). Also, when acetate was compared with a protein-rich wastewater as substrate in MFC, the MFC based on acetate-induced consortia achieved more than 2-fold maximum electric power, and one half of optimal external load resistance compared to the MFC based on consortia induced by a protein-rich wastewater (Liu et al., 2009). However, the protein-rich wastewater being a complex substrate provides the possibility of enriching more diverse microbial community than acetate. Having a more diverse microbial community helps to use various substrates or to convert complex organics to simpler compounds such as acetate which is used as electron donor for current production.

### **2.7.2. Glucose**

Glucose is another commonly used substrate in MFCs. Kim et al. (2000) reported that the performance of a MFC containing *Proteus vulgaris* depended on the carbon source in the initial medium of the microorganism and glucose initiated cells in MFC run for a short time period compared with galactose. Rabaey et al. (2003) re-reported that a maximum power density of  $216 \text{ W}/\text{m}^3$  was obtained from a glucose fed-batch MFC using  $100 \text{ mM}$  ferric cyanide as cathode oxidant. Hu (2008) evaluated the

feasibility of anaerobic sludge as fuel for electricity generation in MFC and compared it with glucose. In a baffle-chamber membrane-less MFC, anaerobic sludge added very limited substrate and a limited power ( $0.3 \text{ mW/m}^2$ ) could be generated. However, with glucose in the same system, a maximum power of  $161 \text{ mW/m}^2$  was generated. In another study, the energy conversion efficiency (ECE) of acetate and glucose as substrates in MFC was compared (Lee et al., 2008). The ECE was 42% with acetate, but was only 3% with glucose which led to a low current and power density as well. In a recent study by Chae et al. (2009), glucose-fed MFC generated the lowest CE as a result of electron loss by competing bacteria, but its relatively diverse bacterial structure enabled much wider substrate utilization and the greatest PD. The low CE was due to the fact that glucose is a fermentable substrate implying its consumption by diverse competing metabolisms such as fermentation and methanogenesis that cannot produce electricity. To explain the much wider substrate specificity of the glucose-enriched MFC than the others, Chae et al. (2009) proposed the presence of a more complex mixed consortium of diverse electricigens or their syntrophic bacteria as a result of the production of diverse fermentation byproducts during glucose degradation.

### **2.7.3. Lignocellulosic biomass**

The abundance and renewability of lignocellulosic materials from agricultural residues renders them a promising feedstock for cost-effective energy production (Huang et al., 2008). However, lignocellulosic biomass cannot be directly utilized by microorganisms in MFCs for electricity generation. It has to be converted to monosaccharides or other low-molecular-weight compounds (Ren et al., 2007). Catal et al. (2008a) demonstrated that all monosaccharides that can be directly generated from hydrolysis of lignocellulosic biomass were good sources for electricity generation in MFCs. When cellulose is used as the substrate, electricity generation requires a microbial community with both cellulolytic and exoelectrogenic activities (Rezaei et al., 2009b). Electricity generation in MFCs from corn stover waste biomass using samples prepared through either neutral or acid steam-exploded hydrolysis processes that convert the hemicellulose to soluble sugars was explored by Zuo et al. (2006). Maximum PDs using an air-cathode containing a diffusion layer and increased solution conductivity ( $20 \text{ mS/cm}$ ) were  $371 \text{ mW/m}^2$  and  $367 \text{ mW/m}^2$  for the neutral and acid hydrolysates ( $1000 \text{ mg COD/L}$ ,  $250 \text{ X}$ ). Very recently, the use of raw corn stover as a substrate for electricity generation in a single-chambered

MFC was demonstrated (Wang et al., 2009b) though the power output was much less than that with glucose as substrate. No effective microorganisms for conversion of pentoses (one of the main components in lignocellulose hydrolysates) to bioethanol have been found yet, rendering a large fraction of residual plant material unsuitable for bioethanol production. Using xylose (typical pentose), PD of 69 mW/m<sup>2</sup> was reported at 10 mM concentration which was less than PD for glucose (97 mW/m<sup>2</sup> at same concentration) indicating that xylose is more difficult to utilize for power generation than glucose (Huang and Angelidaki, 2008).

#### **2.7.4. Synthetic wastewater**

Synthetic or chemical wastewater with well-defined composition is also used by several researchers as it is easy to control in terms of loading strength, pH and conductivity. Venkata Mohan et al. (2008a,b) have used synthetic wastewater at different loading rates in similar MFC configurations to achieve variable performances. Several media used for bacterial growth contains significant amount of redox mediators, such as cysteine, and high strength wastewater contains reduced sulfur species, which can work as abiotic electron donor and increase power production for a short while (Aldrovandi et al., 2009) thus not representing the true performance of the system. This can be avoided by using a minimal salt medium with a single electron donor such as glucose or acetate. To check the influence of wastewater composition on the performance of MFC, Rodrigo et al. (2009) fed MFCs with two different synthetic wastewaters with the same organic pollutants (glucose and peptone) and same organic loading (315 mg/dm<sup>3</sup>) but with a different ratio of readily/slowly biodegradable substrate. The MFC fed with slowly biodegradable waste was more efficient in terms of electricity production probably due to the production of intermediates favoring electricity formation.

#### **2.7.5. Brewery wastewater**

Wastewater from breweries has been a favorite among researchers as a substrate in MFCs, primarily because of its low strength. Besides, it is suitable for electricity generation in MFCs due to the food-derived nature of the organic matter and the lack of high concentrations of inhibitory substances (for example, ammonia in animal wastewaters) (Feng et al., 2008). Although the concentration of brewery wastewater varies, it is typically in the range of 3000–5000 mg of COD/L which is approximately 10 times more concentrated than domestic wastewater (Vijayaraghavan et al., 2006). It could also be an ideal substrate for MFCs due to its

nature of high carbohydrate content and low ammonium nitrogen concentration. Beer brewery wastewater treatment using air-cathode MFC was investigated by Feng et al. (2008) and a maximum PD of  $528 \text{ mW/m}^2$  was achieved when 50 mM phosphate buffer was added to the wastewater. In this case the maximum power produced by brewery wastewater was lower than that achieved using domestic wastewater, when both wastewaters were compared at similar strengths. This might be due to difference in conductivities of two wastewaters. Diluting the brewery wastewater with deionized water decreased the solution conductivity from  $3.23 \text{ mS/cm}$  to  $0.12 \text{ mS/cm}$ . Recently, Wen et al. (2009) using a model based on polarization curve for the MFC, reported that the most important factors which influenced the performance of the MFC with brewery wastewater were reaction kinetic loss and mass transport loss (both were  $0.248 \text{ V}$  when current density was  $1.79 \text{ A/m}^2$ ). These can be avoided by increasing the concentration of brewery wastewater and by increasing the reaction temperature and using a rough electrode to provide for more reaction sites.

#### **2.7.6. Starch processing wastewater**

Starch processing wastewater (SPW) contains a relatively high content of carbohydrates ( $2300\text{--}3500 \text{ mg/L}$ ), sugars ( $0.65\text{--}1.18\%$ ), protein ( $0.12\text{--}0.15\%$ ) and starch ( $1500\text{--}2600 \text{ mg/L}$ ), representing an important energy-rich resource, which can be potentially converted to a wide variety of useful products (Jin et al., 1998). SPW was used as a fuel to enrich a microbial consortium generating electricity and current generation ( $0.044 \text{ mA/cm}^2$ ) was coupled to a fall in COD from over  $1700 \text{ mg/L}$  to  $50 \text{ mg/L}$  in 6 weeks (Kim et al., 2004). Lu et al. (2009) operated a MFC with SPW containing  $4900 \text{ mg/L}$  of COD over four cycles and obtained a maximum voltage output and power density of  $490.8 \text{ mV}$  and  $239.4 \text{ mW/m}^2$  in the third cycle. However, the CE was only 7%. They attributed this low CE to oxygen diffusion to the anode compartment resulting inoxidization of other electron acceptors, biomass production and fermentation.

#### **2.7.7. Dye wastewater**

Azo dyes constitute the largest chemical class of synthetic dyes and are extensively present in effluent from dye-manufacturing industries and textile industries. Their removal from these effluents before discharge is of paramount importance as the intense color of these dyes leads to severe environmental problems such as obstruction of light and oxygen transfer into water bodies which in turn is detrimental to aquatic life (Pant et al., 2008). Besides, several of these dyes are also

toxic in nature. Very recently, efforts have been made to utilize these dyes as a substrate in MFC leading to color removal from such dye-containing wastewaters as well as generating electricity. Sun et al. (2009a) reported accelerated decolorization of active brilliant red X-3B (ABRX3), a model azo dye, in a MFC when glucose and confectionary wastewater were used as co-substrates. Though higher dye concentrations (even up to 1500 mg/L) did not inhibit their decolorization; however, electricity generation from glucose was affected by higher concentrations of ABRX3 (>300 mg/L). This was attributed to the competition between azo dye and the anode for electrons from carbon sources. Thus, simultaneous treatment of azo dye-containing wastewater and readily biodegradable organic matter-containing wastewater could be achieved by mixing two kinds of wastewater in the MFCs, with the advantage of saving both cost and energy, however, the system still requires considerable improvements in terms of finding appropriate bacterial community that is capable of utilizing a mixture of dyes and other simple carbon sources in order to make MFCs a realistic solution for this kind of wastewater.

#### **2.7.8. Landfill leachates**

Landfill leachates are heavily polluted landfill effluents with a complex composition containing four major groups of pollutants: dissolved organic matter, inorganic macro-components, heavy metals, and xenobiotic organic compounds (Kjeldsen et al., 2002). The use of landfill effluent in a biological fuel cell for COD removal was first reported by Habermann and Pommer (1991), though no current production values were mentioned. An upflow air-cathode MFC generating electricity continuously from leachate for 50 h was reported by Zhang et al. (2008) with maximum volumetric power  $12.8 \text{ W/m}^3$  obtained at a current density of  $41 \text{ A/m}^2$ . Recently, Greenman et al. (2009) demonstrated that it is possible to generate electricity and simultaneously treat landfill leachate in MFC columns. Gálvez et al. (2009) operated three MFCs fluidically connected in series for simultaneous leachate treatment and electricity generation.

#### **2.7.9. Cellulose and chitin**

Particulate substrates like cellulose and chitin are cheap and readily available biopolymeric materials which can be used for electricity generation. These renewable substrates also form a major component of organic matter in industrial and municipal wastewaters (Rezaei et al., 2009a). There have been only a few studies on use of particulate substrates in MFCs. For direct conversion of cellulose to electricity in

MFC, the microorganism(s) must be able to hydrolyze cellulose anaerobically and be electrochemically active, utilizing anode as an electron acceptor while oxidizing metabolites of cellulose hydrolysis. PD up to 55 mW/m<sup>2</sup> using cellulose as the substrate and cattle rumen microorganisms as the catalyst have been reported (Rismani-Yazdi et al., 2007). Later, Ren et al. (2008) reported a power density of 153 mW/m<sup>2</sup> using carboxymethyl cellulose as substrate. Very recently, Rezaei et al. (2009a) tested the effect of particle size on maximum power, power longevity and CE using different sized chitin particles. These authors reported that the maximum PD was lower for the largest (0.78 mm) particles (176 mW/m<sup>2</sup>), with the higher PD for the 0.28 mm (272 mW/m<sup>2</sup>) and 0.46 mm (252 mW/m<sup>2</sup>) particle sizes. Thus, using a solid substrate such as cellulose or chitin, the power production is limited due to a low rate of hydrolysis of the particulate material.

#### **2.7.10. Inorganic and other substrates**

Apart from these above mentioned substrates, some other substrates have also been explored. Electricity generation with anodic sulfide oxidation was reported (Rabaey et al., 2006) with a PD of 39 mW/L. Huang and Logan (2008) reported the effectiveness of electricity production with paper recycling plant wastewater using MFC and obtained a maximum PD of 672 mW/m<sup>2</sup> after amending the wastewater with phosphate buffer. However, with unamended wastewater, the power output was only 144 mW/m<sup>2</sup> mainly due to low solution conductivity. Luo et al. (2009) reported the degradation of phenol and current generation in MFC. The power generation using phenol as the sole substrate was lower than that of glucose and the CE was less than 10% indicating a substantial loss. The large amount of wastewater produced in integrated biorefineries is also a potential source of energy (Kaparaju et al., 2009). Recently the use of MFCs to remove the fermentation inhibitors in cellulosic biorefineries including furfural, 5-hydroxymethylfurfural, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxyacetophenone while simultaneously producing electricity was demonstrated (Borole et al., 2009). A combination of a carbon monoxide (CO) fermenter and MFC as an anaerobic continuous process was also reported recently (Kim and Chang, 2009). The CO fermenter was enriched to produce acetate which was fed to a MFC to generate electricity. Though the conversion yield was quite low, it proved that syn-gas (mainly CO) can be converted to electricity through microbial process. 1,2-Dichloro ethane degradation by anodophilic bacteria enriched in MFCs was reported by Pham et al. (2009a). Further,

removal of sulfate and thiosulfate in a single- chamber MFC inoculated with *Desulfovibrio desulfuricans* was investigated (Zhao et al., 2009) and a maximum current production of  $0.115 \text{ mA/cm}^2$  was observed. 4. Current and power outputs achieved in MFCs using different substrates. The production of current in an MFC is directly linked to the ability of the bacteria to oxidize a substrate and transfer electrons resulting from this oxidation to the anode electrode. The current and PD, CE and pollutants removal efficiencies differ between the various studies according to the experimental conditions (initial wastewater composition, concentration, and MFC set up conditions). Table 1 presents the current density ( $\text{mA/cm}^2$ ) at maximum power density ( $\text{W/m}^2$ ) achieved using various substrates in MFCs. With similar designs of MFC,  $506 \text{ mW/m}^2$  was produced with acetate (Liu et al., 2005), but  $261 \text{ mW/m}^2$  with swine wastewater (Min et al., 2005) and  $146 \text{ mW/m}^2$  with domestic wastewater (Liu and Logan, 2004). The maximum power density produced appears to be related to the complexity of the substrate (i.e. single compound versus several compounds). Heilmann and Logan (2006) reported that with substrates like peptone and meat processing wastewater containing many different amino acids and proteins, lower power was produced than achieved using single compound like bovine serum albumin (BSA). The power generation measured using xylose as substrate was lower than studies with other fuels such as acetate or glucose (Huang et al., 2008). However, the fact that xylose bioconversion in MFCs takes place at room temperature and relatively low substrate concentration levels, whereas anaerobic digestion generally fails due to low reaction rates, may make the MFC a complementary technology to the anaerobic digestion for celluloses and its hydrolytes (Pham et al., 2006). Recently, while evaluating the potential of various eco-systems in harnessing bioelectricity through benthic fuel cells, Venkata Mohan et al. (2009) reported that the substrate concentration of the water body showed significant influence on the power generation as they act as carbon source (electron donor) for the benthic metabolic activity. Water bodies containing higher organic matter were able to generate higher power output. The beginning 10 years of research on MFCs have resulted in a 10,000-fold increase in the current density obtained from MFCs (Rabaey et al., 2004). This has further improved in recent years. Nevin et al. (2008) reported that *G. sulfurreducens* grown on acetate produced  $2.15 \text{ kW/m}^3$  anode volume, which is the highest MFC power density reported to date. Similarly, a new axenic strain *Rhodopseudomonas palustris* DX-1, isolated from an



MFC produced higher power output ( $2720 \text{ mW/m}^2$ ) than other mixed cultures (Xing et al., 2008). However, at present the power generated by MFCs is low from the view of large-scale wastewater treatment. In fact the only MFC type that has been used for practical applications is sediment MFCs which harvest power from sediment by embedding an anode in sediment and connecting it via an electrical circuit to a cathode placed in the overlying aerobic seawater, making it feasible to power on-site to sensors and telemetry devices in remote oceanic areas (Tender et al., 2008). It is expected that with time, given the continued interest and support for this research, the output will reach a usable level for other applications as well.

## **2.8. MFC Configurations**

### **2.8.1. Single compartment MFCs**

A useful and simple design for examining factors that affect power production in MFCs was an air-cathode reactor developed at Penn State (*Liu and Logan 2004*). This the single-chamber, air-cathode cube reactor has been used and reported on in over a dozen publications. The simple design of the reactor allows examination of a variety of factors on power production. The cube reactor consists of a single 4-cm block of Acrylic or Lexan (a material that can be autoclaved) drilled through producing a 3-cm-diameter chamber. The empty bed volume is 28 mL, and when the two electrodes are placed on opposite ends the surface area per volume of the reactor (based on the anode projected surface area) is  $25 \text{ m}^2/\text{m}^3$ . Two openings are made on the top to allow the reactor to be easily drained and filled. These are sealed with thick stoppers to prevent oxygen from entering the reactor when the reactor is operated. The electrodes sit in the ends that are cut to be slightly recessed, a round rubber gasket placed over the electrode, and then the flat plate ends are attached on top of the gasket to form a water tight seal. In the first tests the anode was made of Toray carbon paper (without wet proofing; E-Tek, USA; no catalyst), although in later tests other materials were used. The Microbial Fuel Cells cathode is typically carbon cloth containing  $0.5 \text{ mg/cm}^2$  of Pt catalyst (E-Tek, USA) facing the water-side of the reactor. In the first system developed this was covered by a cation exchange membrane (CEM) of Nafion<sup>TM</sup> 117 (Dupont, USA). The CEM was hot pressed directly onto the cathode by heating it to  $140^\circ\text{C}$  at 1780 kPa for 3 min. In other tests rigid carbon paper was used for the cathode that contained  $0.35 \text{ mg/cm}^2$  of Pt (E-Tek, USA). Two pieces of platinum (or stainless steel) wire are inserted through drilled

holes that line up with the bottom of the recessed opening so that good contact is made when the electrode is inserted and pressed onto the wire. Four screw bars are then used to compress the end plates onto the ends, holding the reactor together. The circuit is completed with an external resistor, usually 500 or 1000  $\Omega$ .

### **2.8.2. Two compartment MFCs**

The simplest MFC design consists of two chambers separated by a cation exchange membrane (CEM). Many researchers use such systems to investigate power production (Bergel et al. 2005; Bond et al. 2002; Min et al. 2005a; Oh and Logan 2005; Oh et al. 2004), but there are few photographs of these systems in the literature. The distinguishing features of these systems are two chambers, the CEM, and a cathode suspended in water that is sparged with air. The concentration of dissolved oxygen can affect performance (Oh et al. 2004), with power decreasing as dissolved oxygen is lowered or increasing if pure oxygen is used. The anode chamber can also be sparged with gas (for example, with nitrogen to remove oxygen), although some bacteria may be adversely affected by the shear created by the gas bubbling. The chambers can be mixed with a stir bar to maintain homogeneous conditions in the reactor.

### **3. MATERIALS AND METHODS**

In this work, the production of electricity and the oxidation of the pollutants contained in a synthetic wastewater with starch as the carbon source, using a mediator-less two-compartment microbial fuel cell (MFC) has been studied

Oxygen in the anode chamber inhibits electricity generation, so the two compartment MFC system must be designed to keep the microorganisms separated from oxygen. This separation of the bacteria from oxygen can be achieved by placing a membrane that allows charge transfer between the electrodes, forming two separate chambers: the anode chamber, where the bacteria grow; and the cathode chamber, where the electrons react with the electron acceptor. The cathode was sparged with air to provide dissolved oxygen for the reaction. The two electrodes were connected by a wire containing a load. In principle, the membrane is permeable to protons that are produced at the anode, so that they can migrate to the cathode where they can combine with electrons transferred via the wire and with oxygen, forming water. The current produced by MFC was simply calculated in the laboratory by monitoring the voltage drop across the resistor using a multimeter connected up to a computer for continuous data acquisition.

The preliminary works, acclimation period and start-up of MFC, were done to study the production of electricity and oxidation of the pollutants in synthetic wastewater having with starch as carbon source.

#### **3.1. The Preliminary Work for the Setup of the MFC System**

##### **3.1.1. Acclimation period**

Activated sludge was taken from Bahçeşehir Domestic Wastewater Treatment Plant and fed with starch solution mixture in an aerated fill and draw reactor. The glass reactor which has an effective volume of 4 L was used. Figure 3.1 shows the acclimation reactor.



**Figure 3.1:** The acclimation reactor.

The hydraulic detention time was set at one day, and the aeration of the reactor was stopped after 23 hours to allow one hour of settling. The supernatant was wasted after settling and the reactor was filled with aerated tap water and fed with starch solution. The starch solution having a COD concentrations of 600 mg/L was prepared weekly.

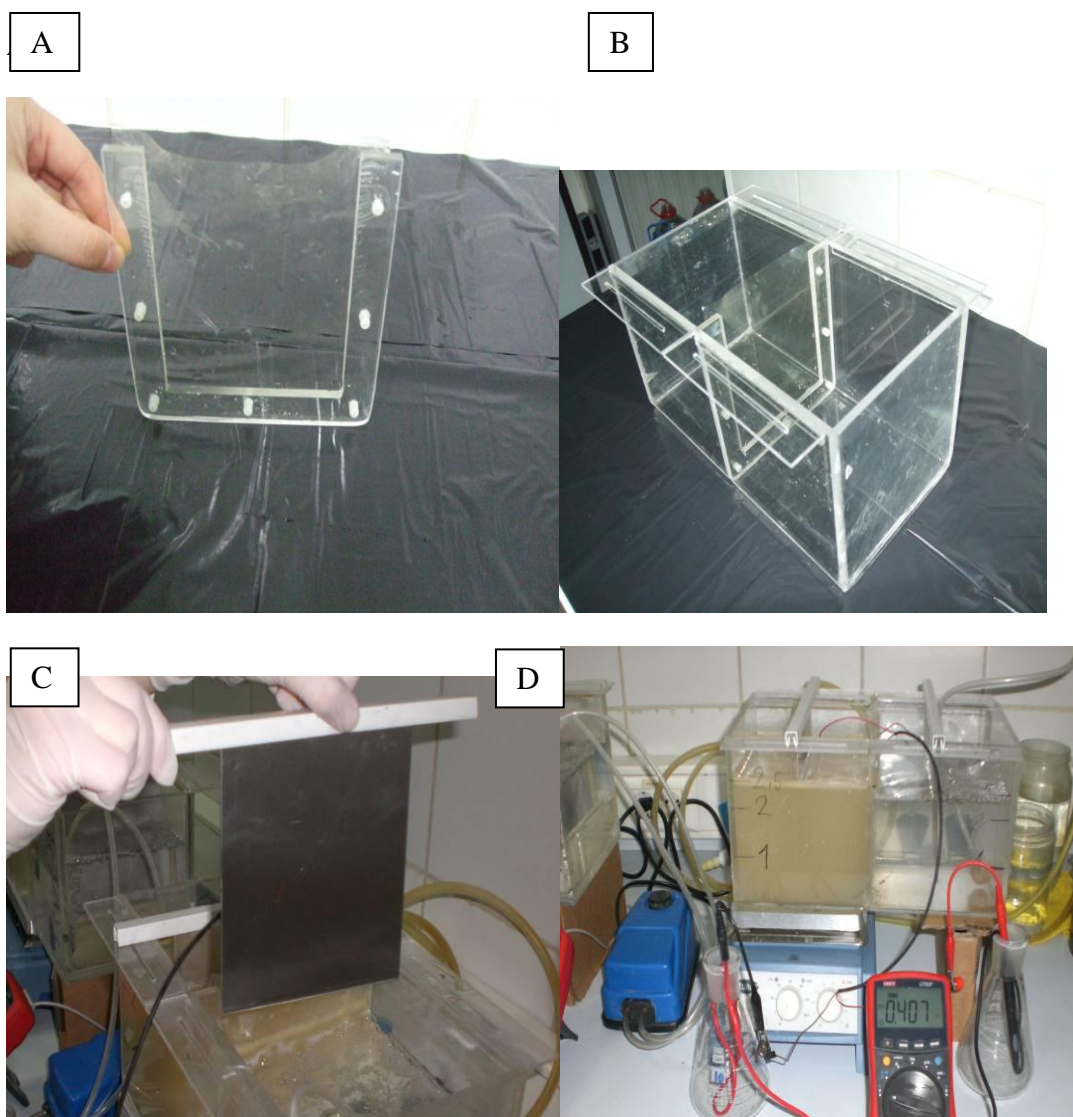
After the mixed liquor MLVSS concentration reached the desired level of 2000 mg/L, the daily MLVSS concentrations were measured and the excess sludge produced was wasted. When the amount of excess sludge was approximately constant, the fill and draw system is defined to reach steady-state at a constant F/M ratio with definite sludge age of 10 days and constant daily COD removal efficiency.

### 3.1.2. Start-up period of MFC

#### 3.1.2.1. MFC Design

The constructed microbial fuel cell system consists of the following units:

1. Reactor ( Anode and Cathode Chambers)
2. Proton Exchange Membrane (Nafion 117)
3. Electrodes (Chrome-Nickel Plate)
4. Stirrer
5. Air Pump
6. Multimeter
7. Computer



**Figure 3.2:** The necessary materials for the start-up period of the MFC, (A) Nafion membrane, (B) MFC reactor with Nafion membrane, (C) Electrode, (D) MFC system with the multimeter.

Microbial fuel cell was operated in fill and draw mode at room temperature.

The two-chamber MFC consisted of two plexyglass chambers (15cm×15cm×15 cm) and with a proton exchange membrane (PEM, Nafion 117) separating the reactor into two parts. Volume of the anode and cathode chambers were same and about 2.5 L.

The electrode made of chrome-nickel was placed in both chambers. The surface areas of the anode and cathode were the same, about 225 cm<sup>2</sup>.

Voltage was measured using a multimeter and a data acquisition system, which can continuously monitor the voltage and transfer data to the computer at an interval of 2 min.

### **3.1.2.2. Set-up and start-up operation of the system**

In the set-up period, connections and placements of materials were done in eleven steps. These steps can be listed as follows:

1. A reactor separated into two parts which are anodic and cathodic chambers.
2. Proton exchange membrane, Nafion 117, was put in distilled water for two hours to obtain expanded shape.
3. Nafion membrane was placed between two frames which were made of plexyglass.
4. Nafion was placed between anode and cathode chambers.
5. The volumes of the chambers were measured and marked.
6. The electrode which has a black wiring was put in the anodic chamber.
7. The electrode which has a red wiring was put in the cathodic chamber.
8. Stirrer was placed in the bottom of the anode part of MFC.
9. The diffusers were placed in the cathodic chamber.
10. Multimeter was connected to the red and black wires to complete the circuit.
11. The connection between the computer and multimeter was done for continuous data storage.

After setup of the system the anodic chamber was seeded with starch acclimated activated sludge of about 1000 mg/l VSS, to start-up the MFC system. Tap water was added to cathodic chamber and the air was supplied. Stirrer was turned on. After the

nutrient solutions were added on the biomass, the anodic chamber was fed with synthetic wastewater (starch solution).

In this period, soluble COD and voltage profiles of the system was observed for a period long enough to ensure the depletion of the substrate.

### **3.2. Analysis Conducted and Calculated Parameters in the MFC System**

After start-up period, the anodic chamber of the microbial fuel cell was fed with different concentrations of starch solution, respectively 525 mg/l, 1050 mg/l and 250 mg/l COD, for three different periods. The aerobic cathodic chamber was not stirred but aerated with a sufficiently small flow rate of air, to prevent the crossover of the oxygen from the cathodic to the anodic chamber. This chamber only contained tap water.

The voltage can be defined as a function of the external resistance, or load on the circuit, and the current. The cell voltage of MFC was recorded automatically by a computer once every three minutes.

The highest voltage produced in an MFC is the open circuit voltage (OCV) which was measured with the circuit disconnected (infinite resistance, zero current). OCV was determined for different starch concentrations. After determination of OCV a 1000 $\Omega$  external resistance was connected to the MFC.

The current produced from a MFC was small, so the current was not measured, but instead it was calculated according to Ohm's law,

$$I_{MFC} = \frac{V_{1000\Omega}}{1000\Omega} \quad (3.1)$$

where  $V_{1000\Omega}$  (V) is the measured voltage,  $I_{MFC}$  (A) is the current, and 1000 $\Omega$  is the external resistance.

To make MFCs useful as a method to generate power, it was essential to optimize the system for power production. Power was calculated from a voltage and current as

$$P = I.V \quad (3.2)$$

The power output by an MFC was calculated from the measured voltage across the load and the current as

$$P = I_{MFC} \cdot V_{MFC} \quad (3.3)$$

where P (Watt) is the power,  $I_{MFC}$  (A) is the calculated current, and  $V_{MFC}$  (V) is the measured voltage.

As with any power source, the objective was to maximize power output and therefore to obtain the highest current density under conditions of the maximum potential. Current density was calculated as

$$I_{AN} = \frac{I_{MFC}}{A_{anode}} \quad (3.4)$$

where  $I_{AN}$  (A.cm<sup>-2</sup>) is the anodic current density,  $I_{MFC}$  (A) is the calculated current,  $A_{anode}$  (cm<sup>2</sup>) is the surface area of the used electrode in the anode chamber.

Knowing how much power is generated by an MFC does not sufficiently describe how efficiently that power is generated by the specific system architecture. For example, the amount of anode surface area available for microbes to grow on can affect the amount of power generated. Thus, it is common to normalize power production by the surface area of the anode so that the power density produced by the MFC is given by:

$$P_{AN} = \frac{P}{A_{anode}} \quad (3.5)$$

where  $P_{AN}$  (W.cm<sup>-2</sup>) is the power density, P (W) is the calculated power,  $A_{anode}$  (cm<sup>2</sup>) is the surface area of the studied electrode in the anode chamber.

Polarization curves in MFC give important information about the operating conditions of the MFC, in particular about the actual capabilities of the MFCs. These curves allow discerning three important parameters: the open circuit voltage (OCV) or the maximum allowable MFC voltage (for a nil current), the maximum intensity reachable (for a nil potential) and the maximum feasible current density. Maximum power density and internal resistance of MFCs are obtained by polarization curves. Polarization curves were obtained by varying the external resistance over a range from 10 to 1000Ω when the voltage output achieved is constant. According to Ohm's law, when the power density is the maximum, the internal and external resistances are equal.

While generating power is a main goal of MFC operation, we also seek to extract as much of the electrons stored in the biomass as possible as current, and to recover as



much energy as possible from the system. The recovery of electrons is referred to as *Coulombic efficiency* defined as the fraction (or percent) of electrons recovered as current versus the electrons present in the starting organic matter. The oxidation of a substrate occurs with the removal of electrons, with the moles of electrons defined for each substrate based on writing down the half reaction.

The Coulombic efficiency (CE) was calculated using the ratio of total Coulombs obtained in the experiment (CP) to the theoretical amount (CT) available from complete substrate oxidation.

$$C_E = \frac{M \cdot I_{MFC} \cdot t}{F \cdot n \cdot V_{anode} \cdot \Delta C} \quad (3.6)$$

Where M (g/mol) is molecular weight of the substrate  $I_{MFC}$  (A) represents the calculated current, t (s) is the time interval, F (C/mol) is the Faraday constant (6,485C/mol), n is the number of moles of electrons produced per mol of substrate, V is the volume of anode (L),  $\Delta C$  is the absolute removal amount of COD (g/L)

Chemical oxygen demand and volatile suspended solid were measured in duplicate using standard methods (APHA).

pH was measured using an Orion pH meter .



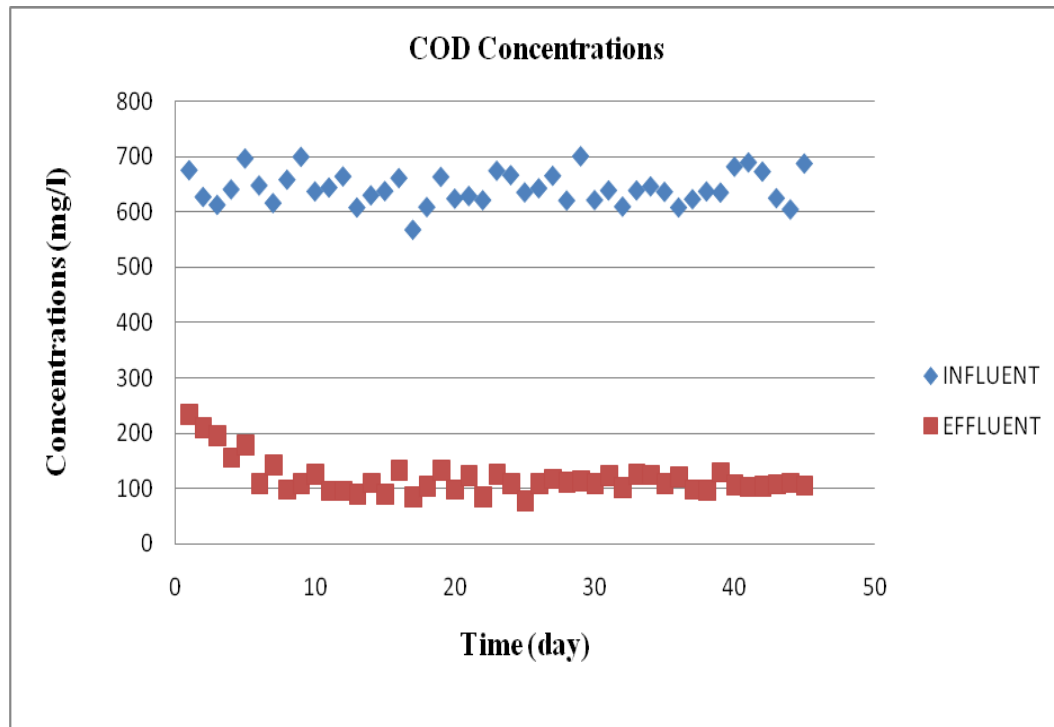
## 4. EXPERIMENTAL RESULTS

### 4.1. The Preliminary Experiment Results

#### 4.1.1. Acclimation period

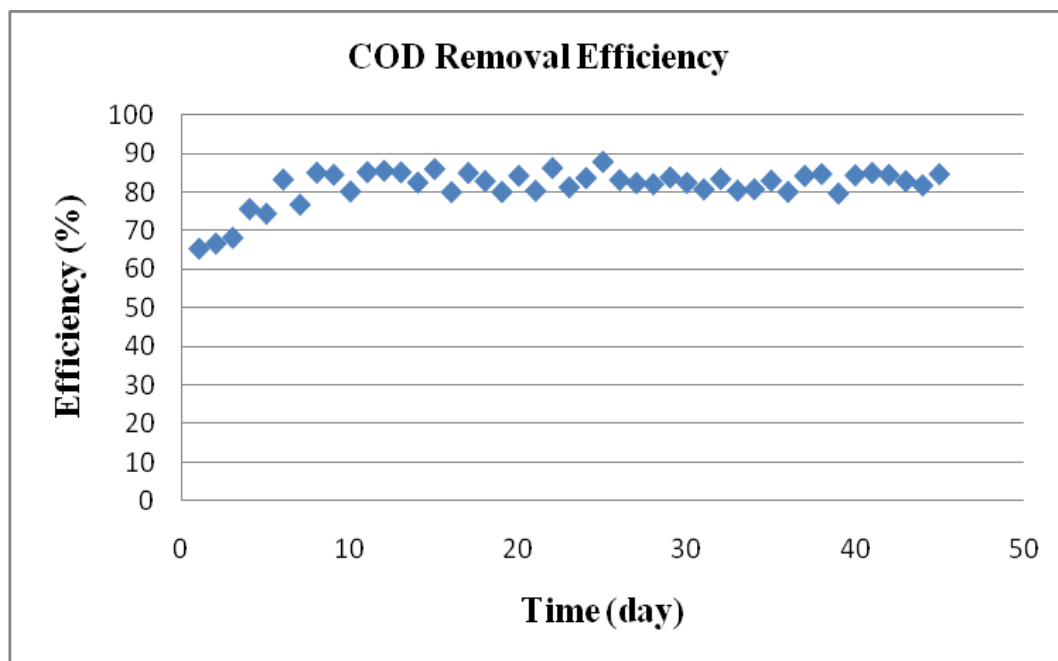
##### 4.1.1.1. COD profiles

During the acclimation period as shown in the Figure 4.1 COD concentrations were decreased each day. COD concentrations were obtained stable after 30 days.



**Figure 4.1:** Influent and effluent COD concentrations in the acclimation reactor.

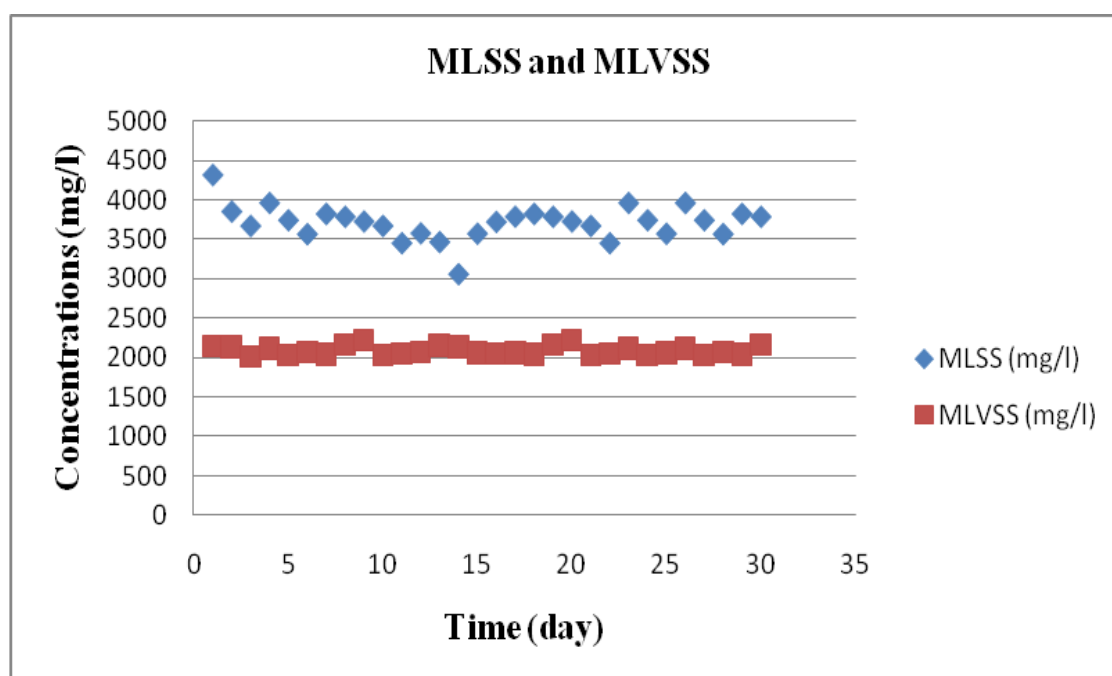
As shown in the Figure 4.2 COD removal efficiencies increased initially and after 30 days COD removal efficiencies was stable. COD removal efficiency reached 88%.



**Figure 4.2:** COD removal efficiency of the acclimation reactor.

#### 4.1.1.2 MLSS-MLVSS profile

MLSS and MLVSS concentrations in the acclimation period are shown in the Figure 4.3. MLVSS concentrations were around 2000 mg/L. The sludge was wasted to keep MLVSS concentrations at 2000 mg/l.

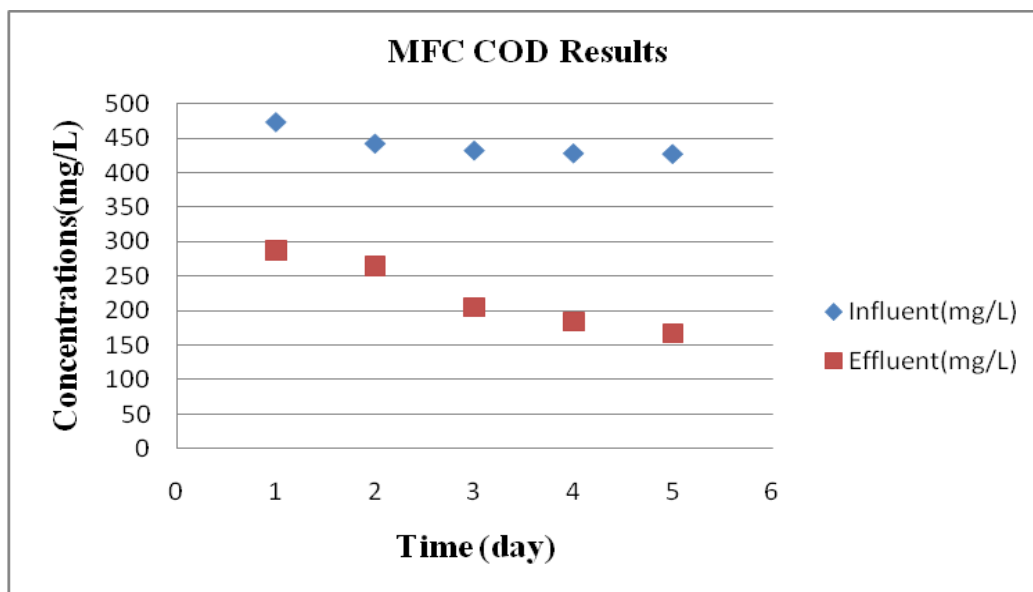


**Figure 4.3:** MLSS-MLVSS concentrations of the acclimation reactor.

### 4.1.2. Start-up Period Results

#### 4.1.2.1. COD profiles

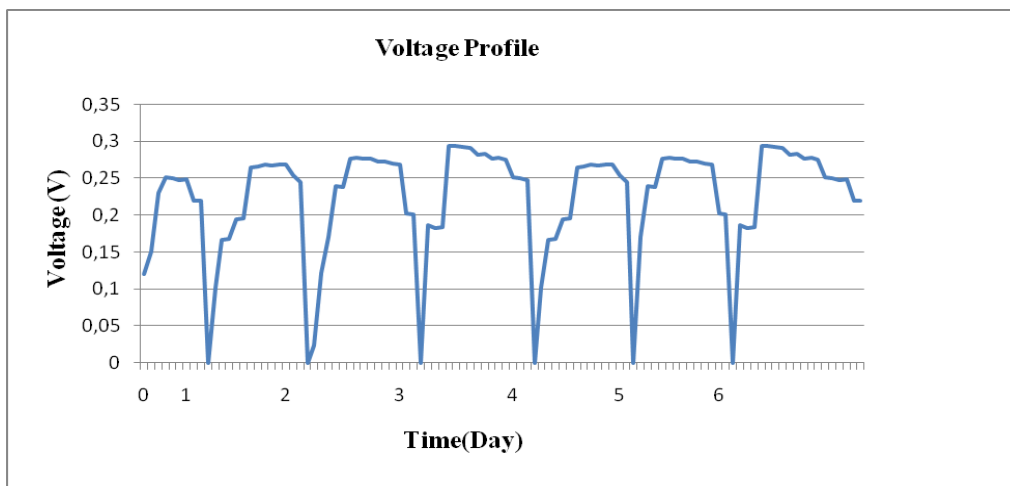
During the start-up period of MFC as shown in the Figure 4.4 effluent COD concentrations decreased each day. The start-up period influent COD was adjusted to 400 mg/L and increasing COD removal was obtained with effluent COD decreasing day by day. The COD removal efficiency reached 75% after 5 days.



**Figure 4.4:** Influent and effluent COD concentrations in the MFC reactor.

#### 4.1.2.2. Electricity generation profile

The electricity generation for the start-up period is given in the Figure 4.5. In this reactor, bacteria in the anode compartment oxidize substrates (electron donors) generating electrons and protons. Electrons are transferred to the cathode through an external circuit, and protons through the internal membrane. It was observed that MFCs could generate electricity directly from hydrolyzable polymeric substrates like starch. At the start-up period when the MFC reactor was fed with 400 mgCOD/L starch solution, electricity generation was observed. When the biomass in the MFC reactor was settled the voltage generation decreased and reached zero eventually. At the feeding time voltage generation shows the noticeable increase. The voltage increased because of biological activity, and stabilized at about 0,25 V for the first 6 days. Following the steady phase, the voltage started to decrease, parallel to the depletion of starch. The supernatant in the anode was replaced and a new cycle was initiated each day.



**Figure 4.5:** Electricity generation in the start-up period.

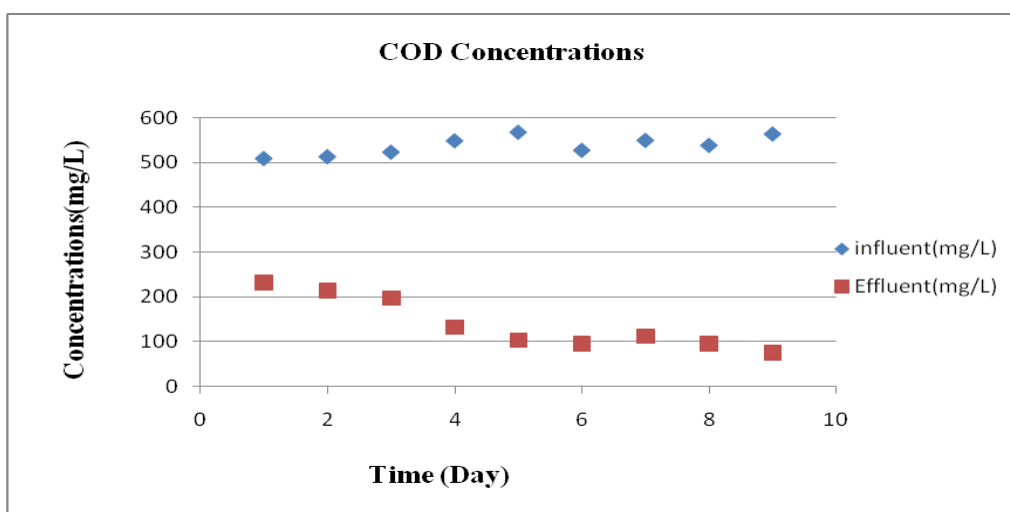
## 4.2. MFC Experiment Results for Different COD Concentrations

### 4.2.1. MFC results for 525 mg/l COD concentration without external resistance

The MFC system was operated feeding 525 mg/L COD concentration with the addition of starch solution. The working volume of the anodic chamber was 2.5 L. All the experiments were performed at room temperature of 25°C in a temperature controlled laboratory.

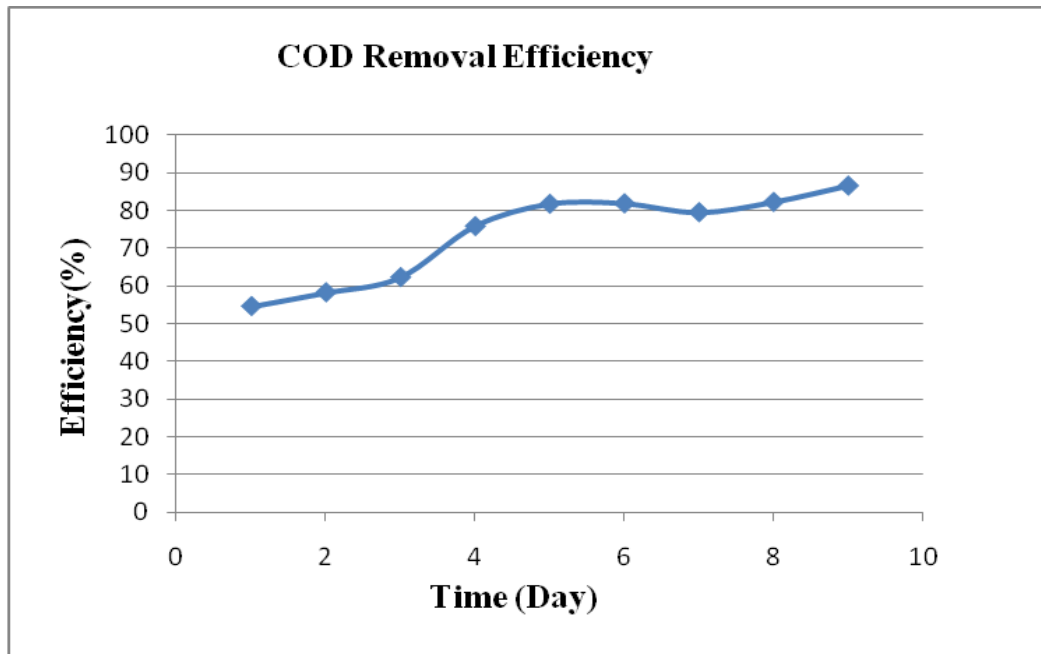
#### 4.2.1.1 COD profiles

The first set of experiments was started feeding the system with 525 mgCOD/L starch solution. As shown in the Figure 4.6 COD concentrations decreased each day and the effluent COD concentration was 75 mg/l after 9 days of operation.



**Figure 4.6:** Influent and effluent COD concentrations and COD removal efficiencies in the MFC reactor.

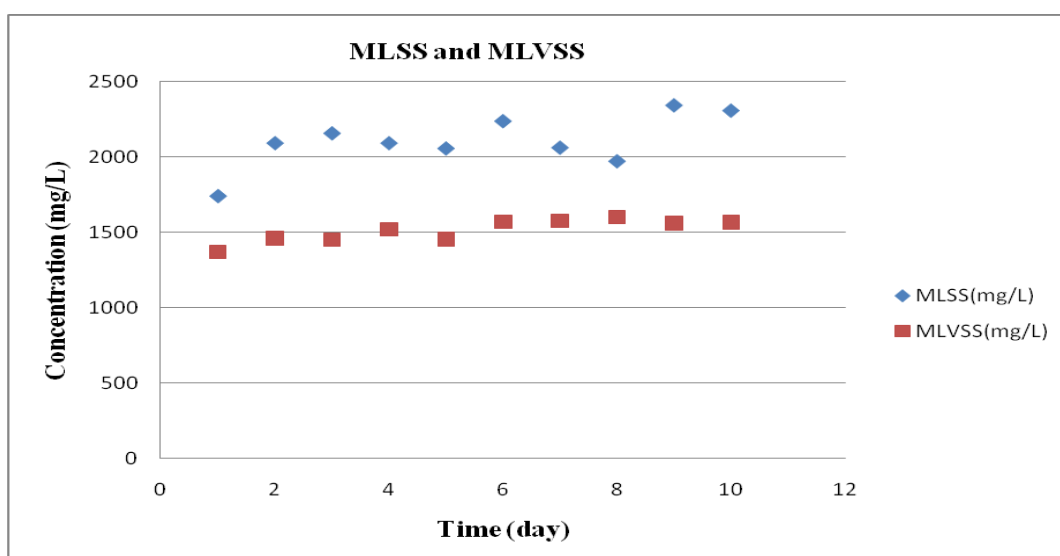
As shown in the Figure 4.7 COD removal efficiencies increased day by day and reached 87% after 9 days of operation.



**Figure 4.7:** COD removal efficiency for MFC experiments.

#### 4.2.1.2 MLSS and MLVSS profiles

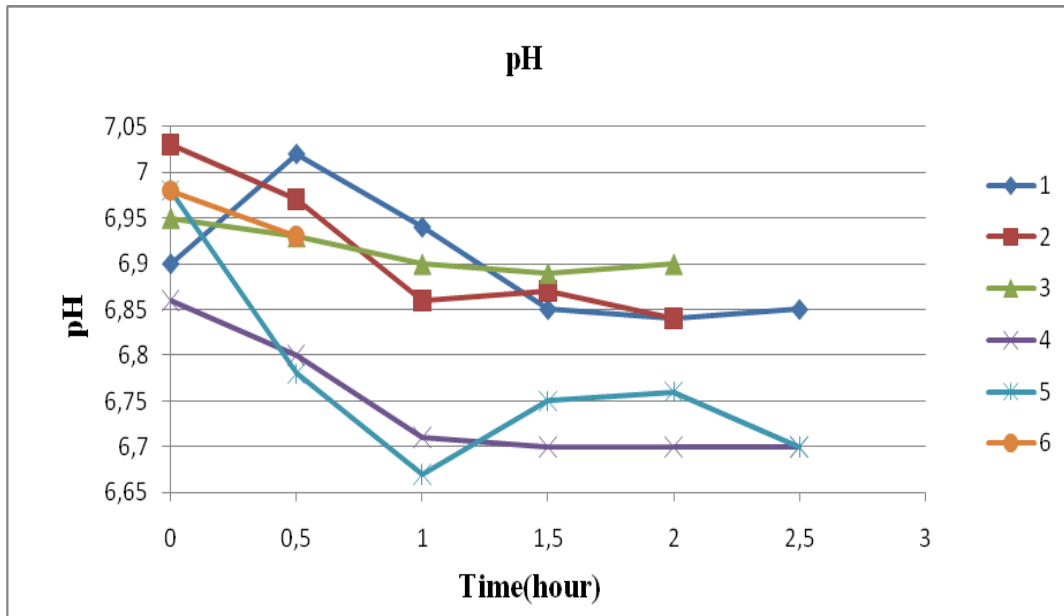
The observed MLSS and MLVSS concentrations are as shown in Figure 4.8. MLVSS concentrations were kept at about 1500 mg/L. The sludge was wasted accordingly. The average daily amount of sludge wasted was 150 ml, exerting a sludge age of 17 days for the MFC system fed with 525 mg COD/L starch.



**Figure 4.8:** MLSS and MLVSS concentrations in the MFC reactor.

#### 4.2.1.3. pH profile

The mixed liquor pH values in the anodic chamber were measured to be between 6.65-7.05 for the first 3 hours after starch feeding, as given in Figure 4.9. The average pH value of the system monitored from time to time always remained in the ranges of 6.5-7.5 throughout the whole cycle.



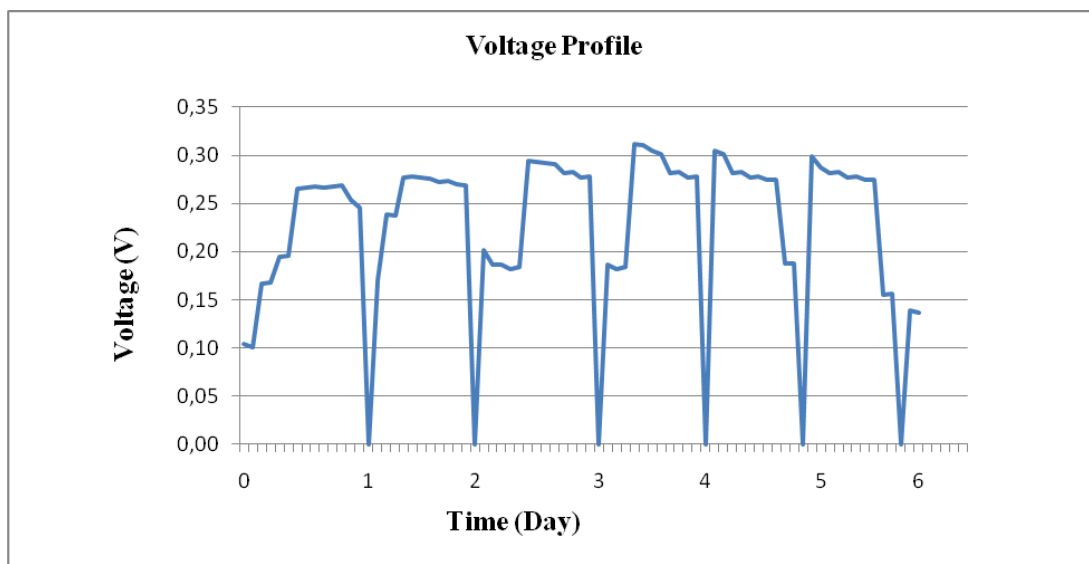
**Figure 4.9:** pH profiles in the MFC reactor.

#### 4.2.1.4. Voltage profile

COD removal and biomass generation took place in parallel with the production of electricity. The electricity generation is seen in the Figure 4.10.

As shown in Figure 4.10, six cycles were monitored for voltage profiles. Electricity generation of each cycle included three phases: ascending phase, stationary phase and declining phase. Data showed higher voltage outputs in the latter cycles than those in the first one. An instantaneous recovery of the voltage generation was observed after the replacement of fresh starch solution in latter cycles, demonstrating the acclimation of biomass to transferring electrons to the metal plate (anode) in the MFC. The system yielded a maximum voltage of 0.3 V just after feeding.

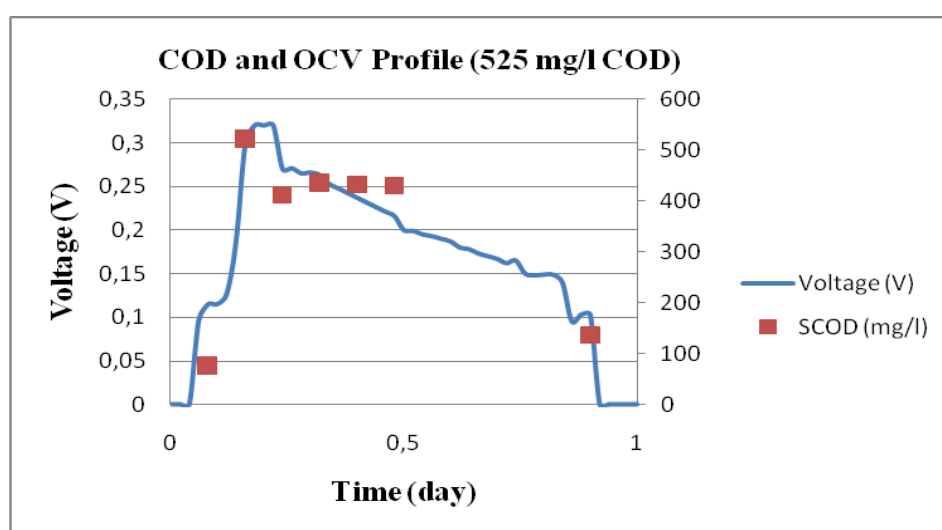




**Figure 4.10:** Voltage profile of the MFC reactor.

#### 4.2.1.5 COD and OCV profile

As seen from the Figure 4.11, COD and voltage profiles were obtained for a day to ensure the depletion of the substrate and OCV in MFC system without resistance. The system was fed with 525 mg/l COD and COD removal was observed for 2 hours after feeding and also after 22 hours. The system was fed with 20 ml Solution A and Solution B for nutritional requirements and to provide a buffer capacity and substrate was added after 30 minutes. The COD removal efficiency was approximately 73% in the MFC reactor. The MFC system fed with starch is said to operated with an OCV of 0.32 V.

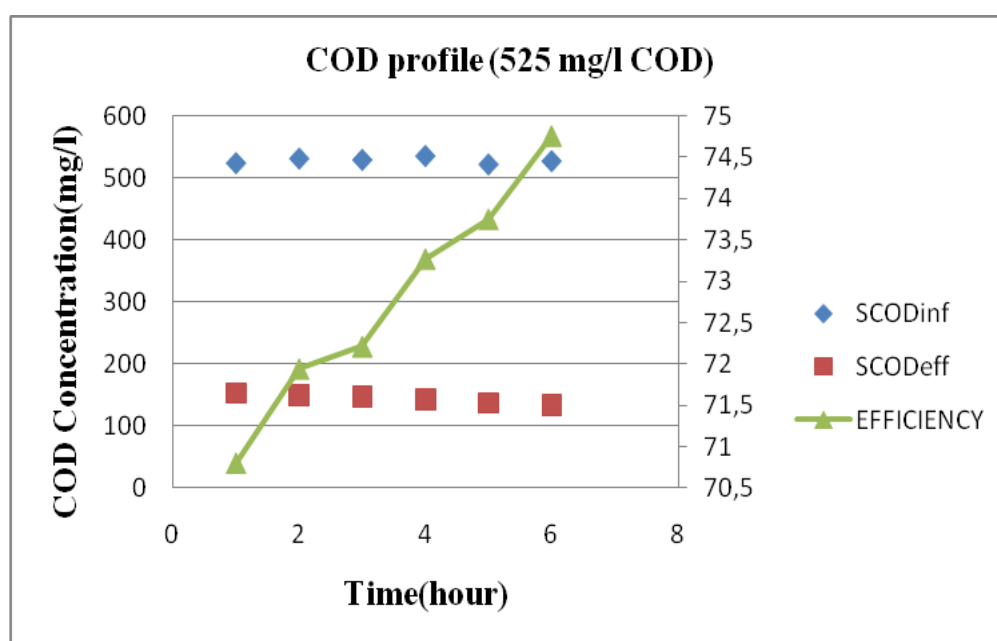


**Figure 4.11.** COD and OCV profiles of the MFC feed with 525 mg/l COD without resistance.

## 4.2.2. MFC results for 525 mg/l COD concentration with external resistance

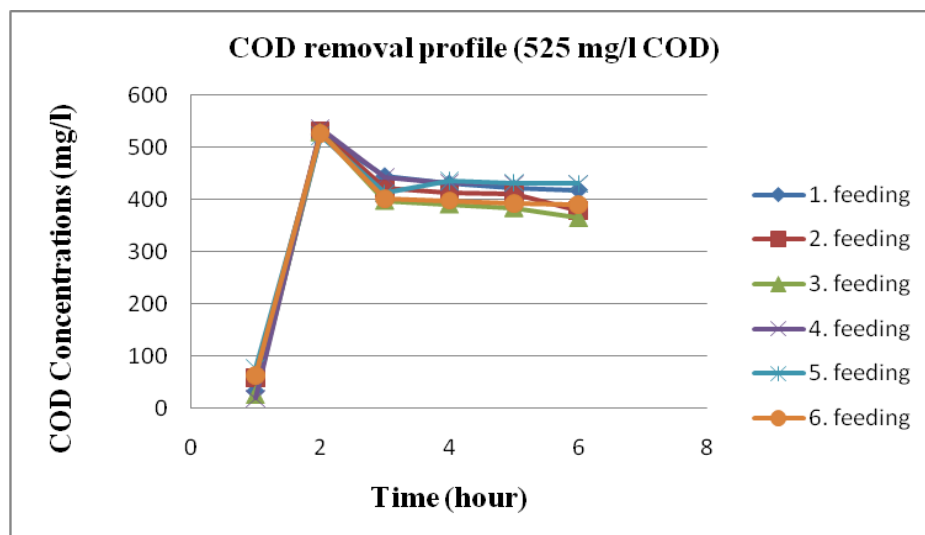
### 4.2.2.1. COD profiles

As seen from the Figure 4.12, soluble effluent and influent COD profiles were obtained for a 6 days to ensure the depletion of the substrate in MFC system with 1 k $\Omega$  external resistance. The system was fed with 525 mg/l COD and COD removal was monitored for 6 days. The COD removal efficiency was approximately 73% for a MFC reactor with external resistance.



**Figure 4.12.** Influent and effluent COD concentrations and COD removal efficiencies of the MFC reactor feed with 525 mg/l COD.

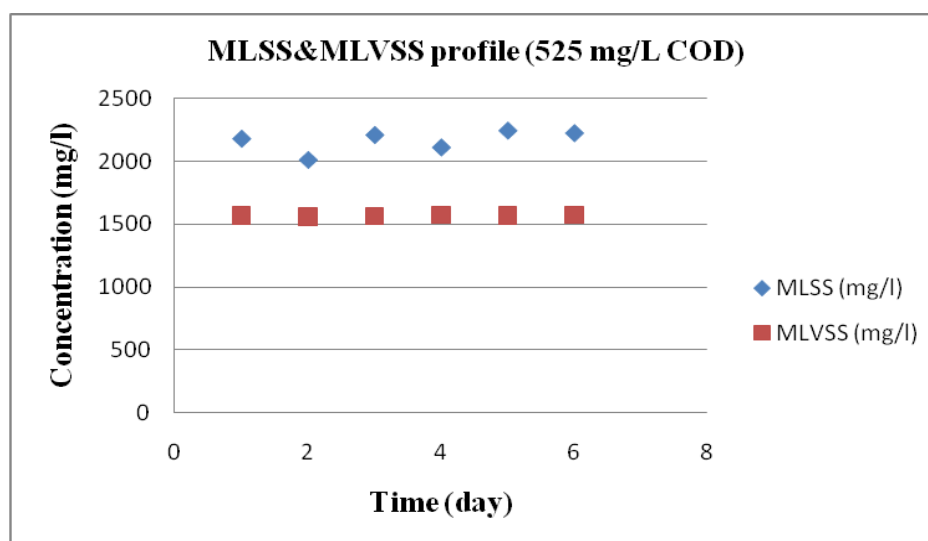
As seen from the Figure 4.13, COD removal of the system during the first two hours after feeding were obtained for a 6 days to ensure the depletion of the substrate in MFC system with 1 k $\Omega$  resistance. More than 20% of COD was removed in 1 hour after feeding. The COD removal efficiencies were similar for all cycles with a slight increase in the last feeding cycles.



**Figure 4.13:** COD removal in the MFC reactor feed with 525 mg/l COD.

#### 4.2.2.2. MLSS and MLVSS profiles

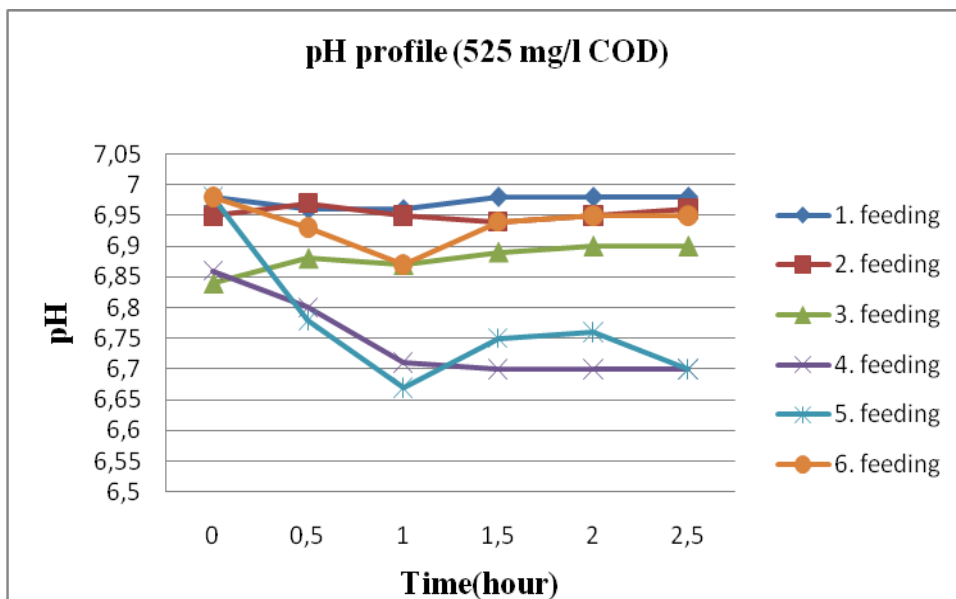
As seen from the Figure 4.14, the mixed liquor MLVSS concentration was 1500 mg/L, the daily MLVSS concentrations were monitored and the average amount of excess sludge wasted was determined as 150 ml/day. During this period, the amount of excess sludge was approximately constant, the system is said to be operated at steady-state with a sludge age of 17 days.



**Figure 4.14:** MLSS-MLVSS concentrations in the MFC reactor feed with 525 mg/l COD.

#### 4.2.2.3. pH profile

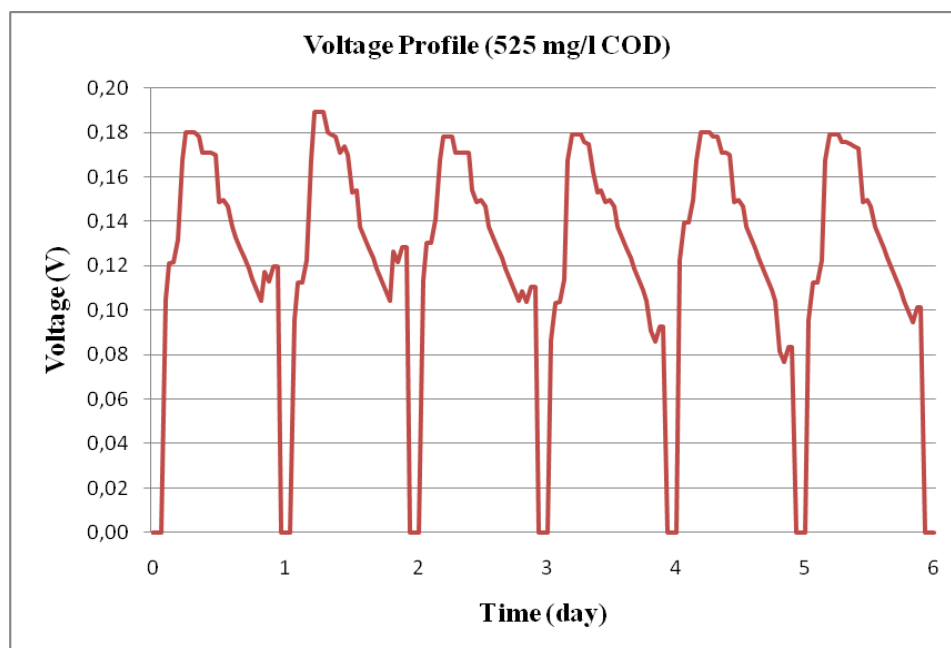
As seen from the Figure 4.15, the pH was approximately between 6.67-6.98 during the monitoring period.



**Figure 4.15:** pH profiles in the MFC reactor feed with 525 mg/l COD.

#### 4.2.2.4. Voltage profile with 1 k $\Omega$ resistance

As seen from the Figure 4.16, the electricity generation in MFC system with 1500 mg/l biomass and 1 k $\Omega$  external resistance was 0.18 V on the average for 6 days of operation. The voltage profiles were similar for each cycle.

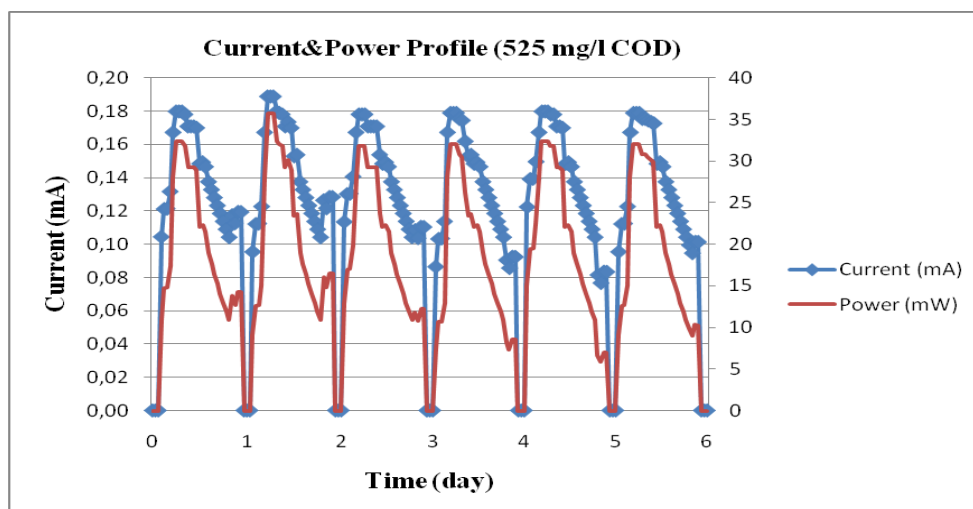


**Figure 4.16:** Voltage profile in the MFC feed with 525 mg/l COD.

#### 4.2.2.5. Power and current profiles

The current and power were calculated as given in Materials and Methods section. As seen from the Figure 4.17, the current and power profiles were obtained for 6 days.

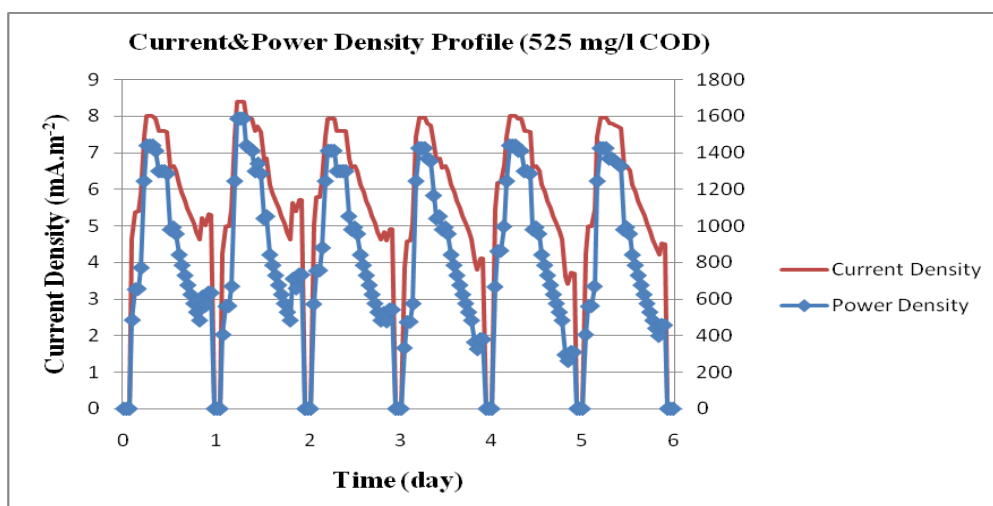
The average current and power obtained during 6 cycles were 0.18 mA and 36 mW respectively.



**Figure 4.17:** Current and power profiles of MFC feed with 525 mg/l COD.

#### 4.2.2.6. Density profiles

The current and power densities were calculated as given in Materials and Methods section. As seen from the Figure 4.18, the current and power profiles were obtained for 6 days. The average current and power densities obtained during 6 cycles were  $7.92 \text{ mA.m}^{-2}$ ,  $1440 \text{ mW.m}^{-2}$ .

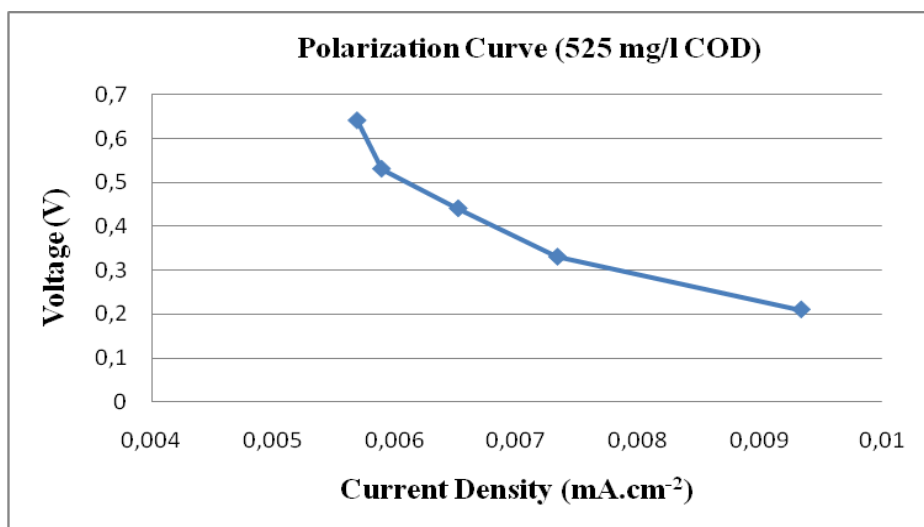


**Figure 4.18:** Current, power density profiles of MFC feed with 525 mg/l COD.

#### 4.2.2.7. Polarization curve

The polarization curve was obtained by plotting voltage versus current density with 5 different external resistances ranging from 1 k $\Omega$  to 5 k $\Omega$  for 525 mg/l feeding. As

seen from the Figure 4.19, when voltage increases, the current density decreases. Internal resistance of the system was calculated from the slope of the curve.

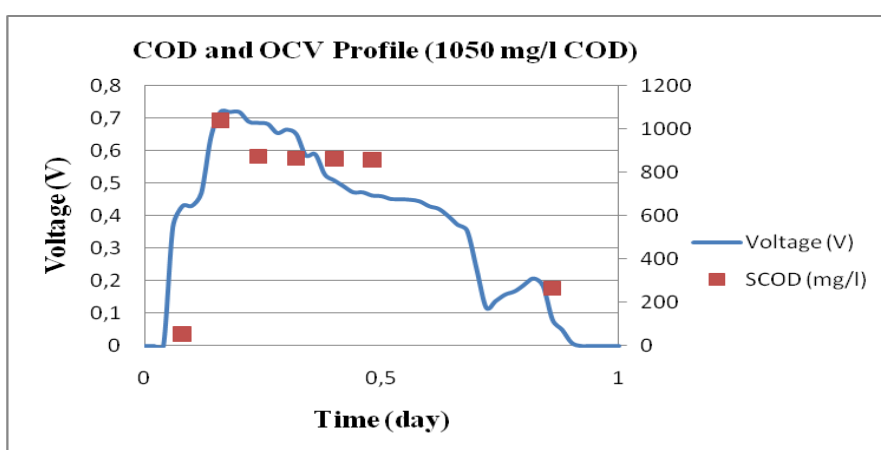


**Figure 4.19:** Polarization curve of the MFC feed with 525 mg/l COD.

### 4.2.3. MFC results for 1050 mg/l COD concentration

#### 4.2.3.1. Voltage and COD profile

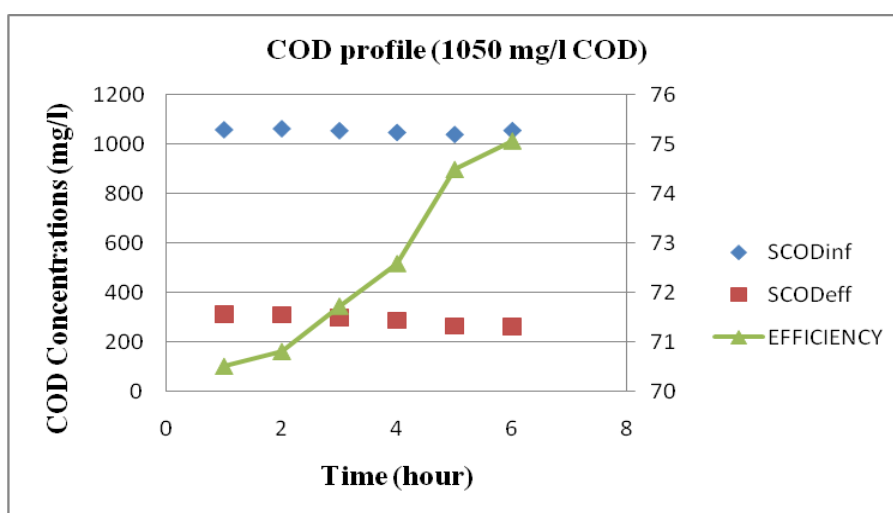
As seen from the Figure 4.20, COD and voltage profiles were observed for a day to ensure the depletion of the substrate and OCV in MFC system without resistance. The system was fed with 1050 mg/l COD and COD removal was observed for 2 hours after feeding. The COD removal efficiency was approximately 73% for a MFC reactor with biomass. MFC system was operated with an OCV of 0.72 V.



**Figure 4.20:** COD and OCV profile of the MFC feed with 1050 mg/l COD without resistance.

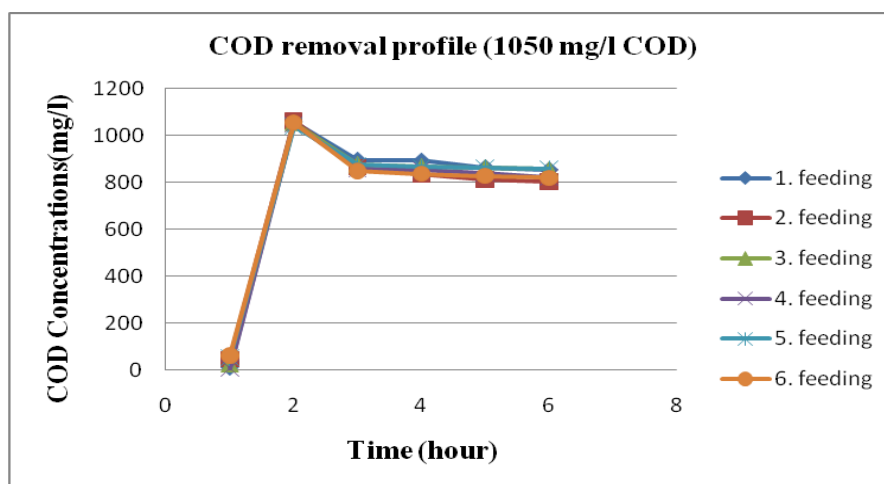
#### 4.2.3.2. COD profiles

As seen from the Figure 4.21, soluble effluent and influent COD profiles were observed for 6 days to ensure the depletion of the substrate in MFC system with 1 k $\Omega$  resistance. The system was fed with 1050 mg/l COD and COD removal was observed for 6 days. The COD removal efficiency was approximately 73% for a MFC reactor with external resistance.



**Figure 4.21:** Influent and effluent COD concentrations and COD removal efficiencies of the MFC reactor feed with 1050 mg/l COD.

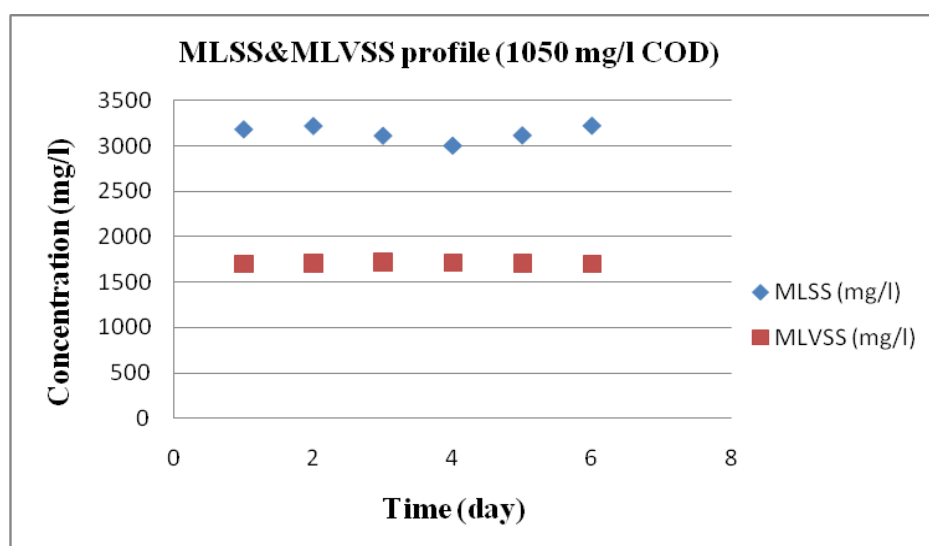
COD removals of the system monitored during two hours after feeding shows that (Figure 4.22), The substrate was depleted in MFC system with 1 k $\Omega$  resistance, similar to the MFC with no external resistance for 6 days. As seen from the graph, more than 20% of the COD was removed after 1 hour feeding.



**Figure 4.22:** COD removal of the MFC reactor feed with 1050 mg/l COD.

#### 4.2.3.3. MLSS and MLVSS profiles

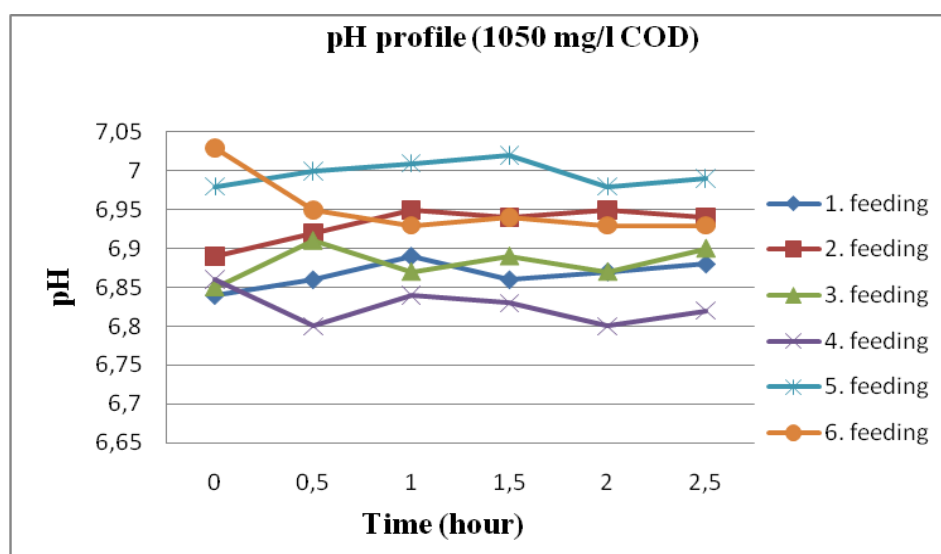
The mixed liquor MLVSS concentration was kept at 1500 mg/L (Figure 4.23). The daily MLVSS concentrations were monitored and the amount of excess sludge produced was determined as 300 mL/day. During this period, the amount of excess sludge was approximately constant and the system is said to be operated at steady-state with sludge age of 8 days.



**Figure 4.23:** MLSS-MLVSS concentrations of the MFC reactor feed with 1050 mg/l COD.

#### 4.2.3.4. pH profile

As seen from the Figure 4.24, the pH in the anodic chamber of the MFC system was approximately between 6.80-7.05.

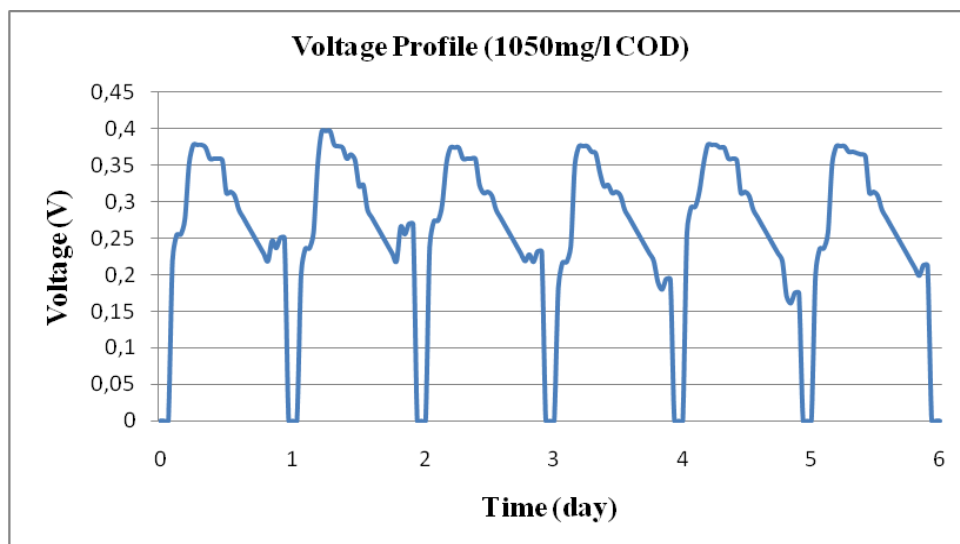


**Figure 4.24:** pH profiles of the MFC reactor feed with 1050 mg/l COD.



#### 4.2.3.5. Voltage profile with suspended biomass and 1 k $\Omega$ resistance

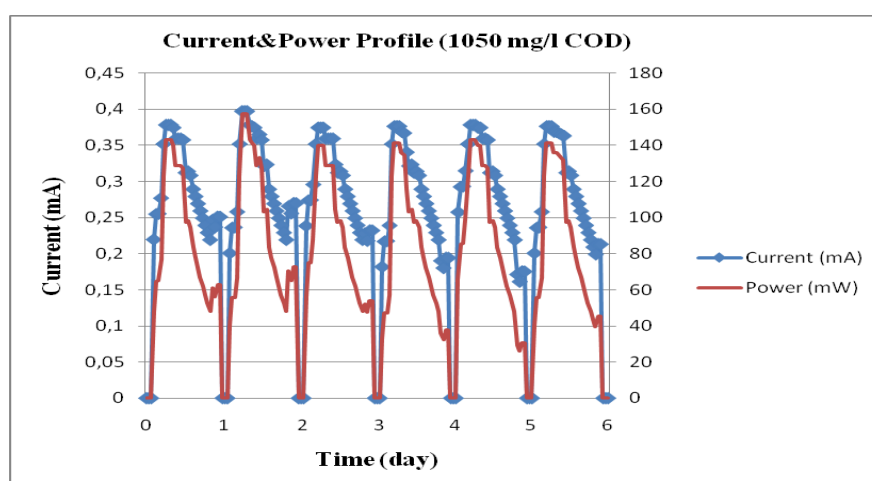
As seen from the Figure 4.25, the electricity generation in the system fed with 1050 mg/L COD, having 1500 mg/L suspended biomass and equipped 1 k  $\Omega$  resistance was stable exerting daily maximum of 0.36 V at beginning of the each cycle.



**Figure 4.25:** Voltage profile of the MFC feed with 1050 mg/l COD.

#### 4.2.3.6. Power and current profiles

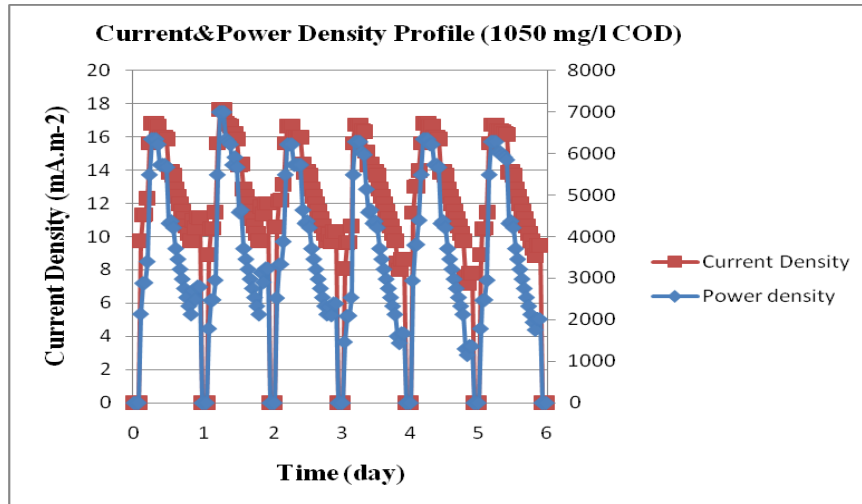
The current and power were calculated as given in the Materials and Methods sections. As seen from the Figure 4.26, the current and power profiles were obtained for 6 days. The average current and power were obtained during as 0.36 mA and the maximum power is 144 mW.



**Figure 4.26:** Current and power profiles of MFC feed with 1050 mg/l COD.

#### 4.2.3.7. Density profiles

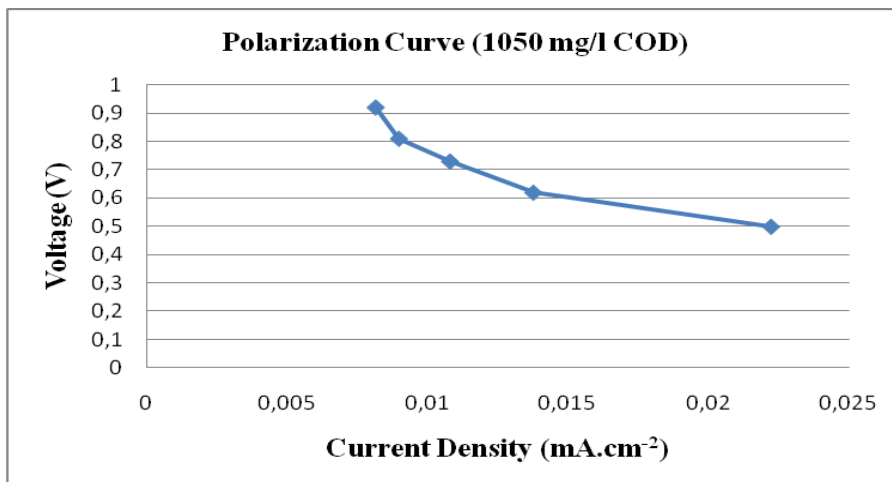
The current and power densities were calculated as given in the Materials and Methods section. As seen from the Figure 4.27, the current and power profiles were obtained for 6 days. The average current and power densities obtained during each cycle were  $17.6 \text{ mA.m}^{-2}$  and  $7000 \text{ mW.m}^{-2}$  respectively.



**Figure 4.27:** Current and power density profiles of MFC feed with 1050 mg/l COD.

#### 4.2.3.8. Polarization curve

The polarization curve was obtained by plotting voltage versus current density with 5 different resistance ranging from  $1 \text{ k}\Omega$  to  $5 \text{ k}\Omega$  for 1050 mg/l COD feeding. As seen from the Figure 4.28, when voltage increases, the current density decreases. Internal resistance of the system was calculated from the slope of the curve.

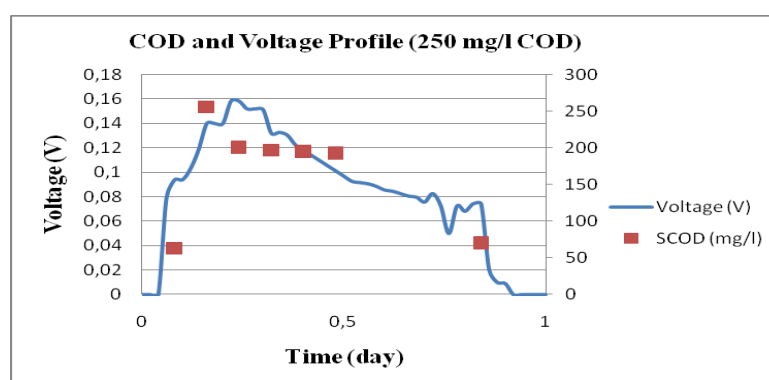


**Figure 4.28:** Polarization curve of the MFC feed with 1050 mg/l COD.

#### 4.2.4. MFC results for 250 mg/l COD concentration

##### 4.2.4.1. Voltage and COD profile

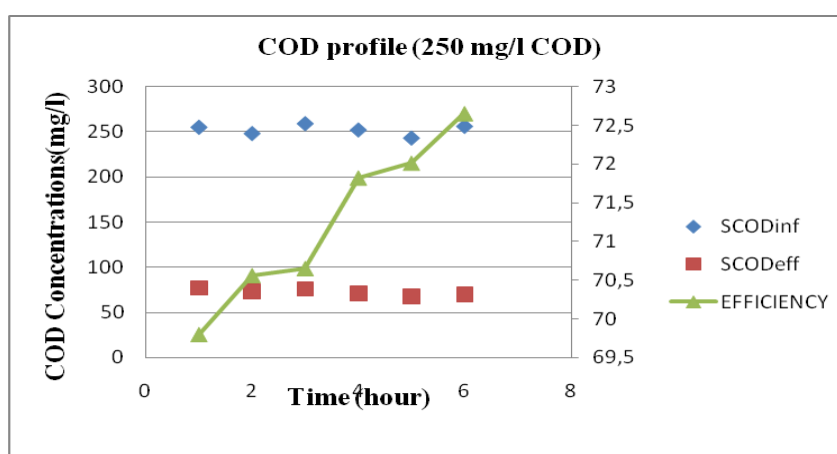
As seen from the Figure 4.29, COD and voltage profiles were obtained for day to ensure the depletion of the substrate and OCV in MFC system without resistance. The system was fed with 250 mg/l COD and COD removal was observed for 2 hours after feeding. The COD removal efficiency was approximately 77% for a MFC reactor with biomass. MFC system was operated with an OCV of 0.14 V.



**Figure 4.29:** COD and OCV profile of the MFC feed with 250 mg/l COD without resistance.

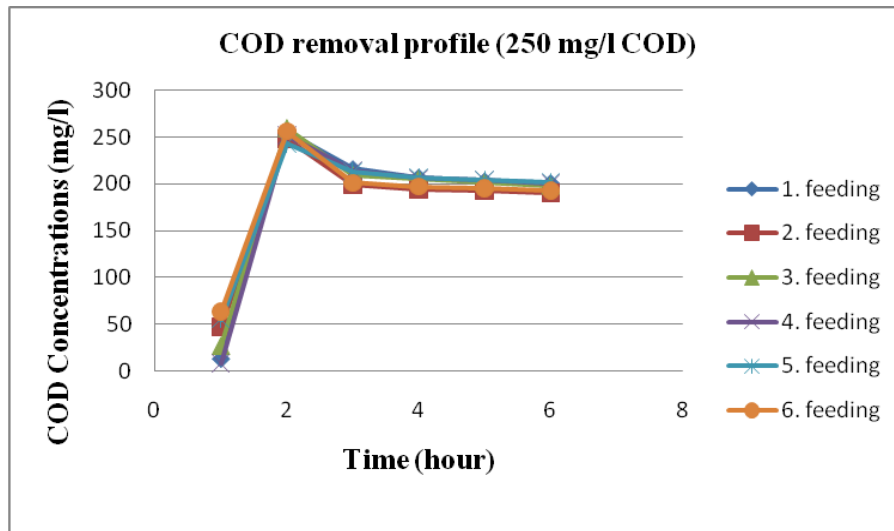
##### 4.2.4.2. COD profiles

As seen from the Figure 4.30, soluble effluent and influent COD profiles were observed for 6 days to ensure the depletion of the substrate in MFC system with 1k $\Omega$  resistance. The system was fed with 250 mg/l COD and COD removal was observed for 6 days. The COD removal efficiency was approximately 72% for a MFC reactor with external resistance.



**Figure 4.30:** Influent and effluent COD concentrations and COD removal efficiencies of the MFC reactor feed with 250 mg/l COD.

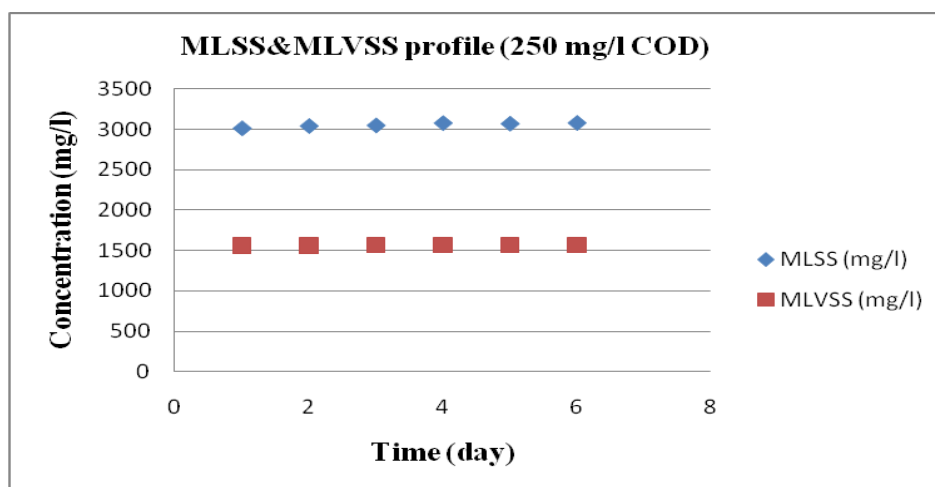
COD removals of the system monitored during two hours after feeding shows that (Figure 4.31), the substrate was depleted in MFC system with 1 k $\Omega$  resistance, similar to the MFC with no external resistance for 6 days. As seen from the graph, more than 20% of the COD was removed after 1 hour feeding.



**Figure 4.31:** COD removal of the MFC reactor feed with 250 mg/l COD.

#### 4.2.4.3. MLSS and MLVSS profiles

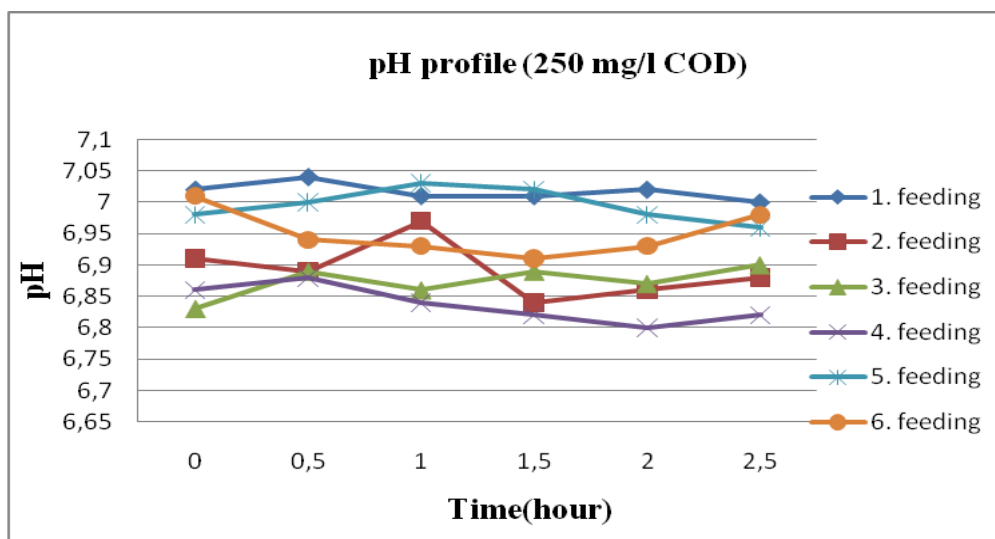
The mixed liquor MLVSS concentration was kept at 1500 mg/L (Figure 4.32). The daily MLVSS concentrations were monitored and the amount of excess sludge produced was determined as 80 mL/day. During this period, the amount of excess sludge was approximately constant and the system is said to was operated at steady-state with sludge age of 31 days.



**Figure 4.32:** MLSS-MLVSS concentrations of the MFC reactor feed with 250 mg/l COD.

#### 4.2.4.4. pH profile

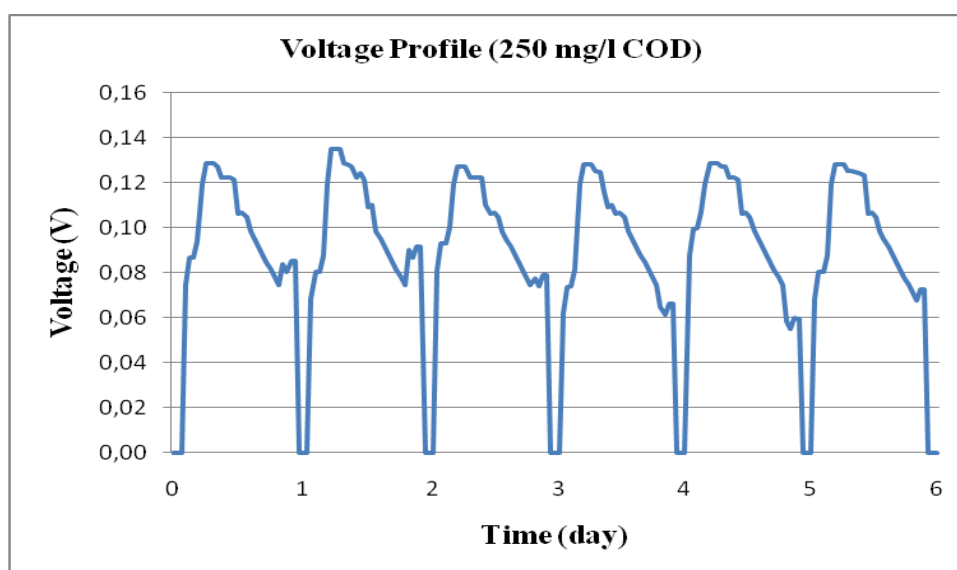
As seen from the Figure 4.33, the pH in the anodic chamber of the MFC system was approximately between 6.80-7.05.



**Figure 4.33:** pH profiles of the MFC reactor feed with 250 mg/l COD.

#### 4.2.4.5. Voltage profile with suspended biomass and 1 k $\Omega$ resistance

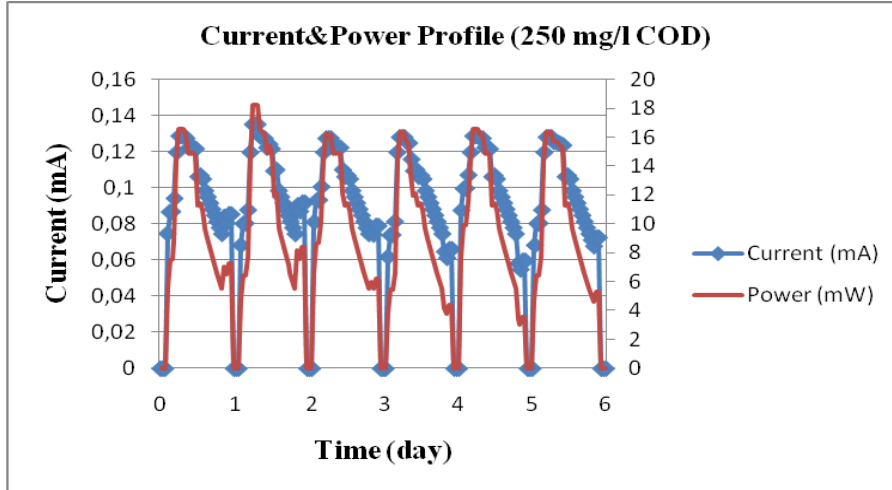
As seen from the Figure 4.34, the electricity generation in the system fed with 250 mg/L COD, having 1500 mg/L suspended biomass and equipped with 1 k $\Omega$  resistance was stable exerting daily maximums of 0.14 V at beginning of each cycle.



**Figure 4.34:** Voltage profile of the MFC feed with 250 mg/l COD.

#### 4.2.4.6. Power and Current profile

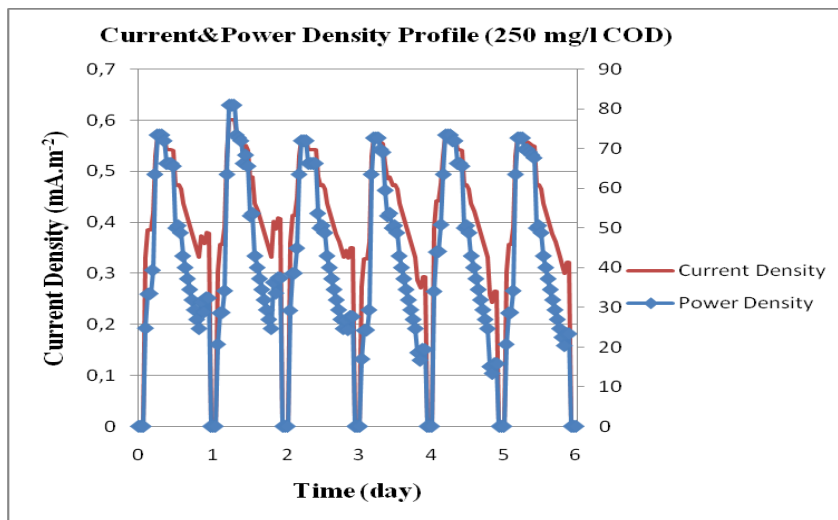
The current and power were given in the Materials and Methods section. As seen from the Figure 4.35, the current and power profiles were obtained for 6 days. The average current and power were obtained as 0.14 mA and the maximum power is 18 mW.



**Figure 4.35:** Current and power profiles of MFC feed with 250 mg/l COD.

#### 4.2.4.7. Density profiles

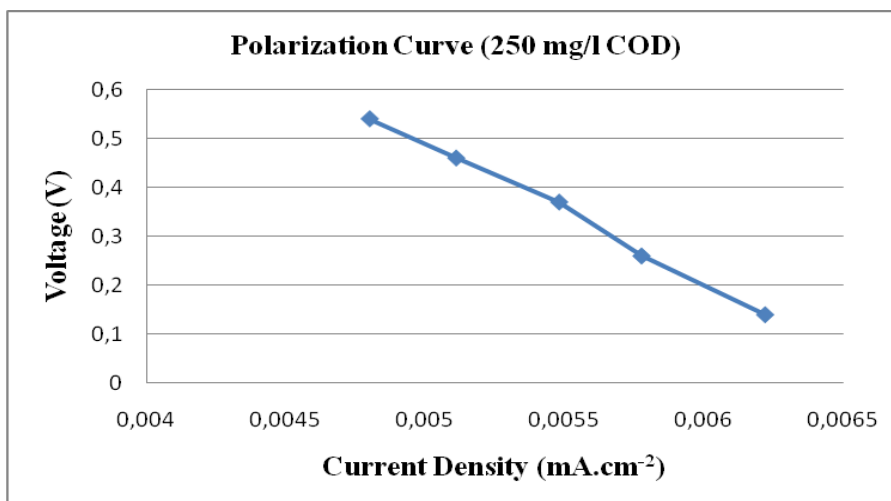
The current and power densities were calculated as given in the Materials and Methods section. As seen from the Figure 4.36, the current and power profiles were obtained for 6 days. The average current and power densities obtained during each cycles were  $5.7 \text{ mA.m}^{-2}$  and  $73.4 \text{ mW.m}^{-2}$  respectively.



**Figure 4.36:** Current, power density profiles of MFC feed with 250 mg/L COD.

#### 4.2.4.8. Polarization curve

The polarization curve was obtained by plotting voltage versus current density with 5 different resistance ranging from 1 k $\Omega$  to 5 k $\Omega$  for 250 mg/l COD feeding . As seen from the Figure 4.37, when voltage increases, the current density decreases. Internal resistance of the system was calculated from the slope of the curve.



**Figure 4.37:** Polarization curve of the MFC feed with 250 mg/l COD.





## **5. RESULT AND DISCUSSION**

This study was conducted to observe the performance of a two compartment MFC fed with synthetic wastewater prepared with starch as the carbon source. The work is focussed on the study of the effect of the biodegradability of starch, paying special attention to the study of the relationship between COD removal and electricity generation, including the achievement of a high power and current density, in the MFC system with anodic and compartment volumes of 2.5 L. The anodic chamber was seeded with 1500 mgVSS/L biomass to simulate the conventional activated sludge systems. The cathodic chamber was filled with tap water and was continuously aerated. The two-compartment MFC with the anodic and the cathodic chambers separated by a proton exchange membrane was used. Carbon removal and electricity generation efficiencies have been observed for different feeding conditions where 250 mg COD/l, 525 mg COD/l, and 1050 mg COD/l was fed to the system. Each set of experiments were run for 1 week with daily cycles of fill and draw operation was applied. The system was monitored for pH and MLVSS changes, and COD removal by the collection of the samples at predetermined intervals. The voltage generation was monitored with a multimeter connected to a computer for continuous data collection.

**Table 5.1:** Overview of the experimental results for the MFC system under different feeding conditions.

Feeding concentration	250 mg/l COD	525 mg/l COD	1050 mg/l COD
Parameters			
MLVSS concentration (mg/l)	1500	1500	1500
F/M ratio (gCOD/gVSS)	0.17	0.35	0.7
Hydraulic Retention Time (day)	2	2	2
Sludge Retention Time (Sludge Age, day)	31	17	8
COD removal (%)	72	73	73
pH	6.80-7.05	6.67-6.98	6.80-7.05
OCV (V)	0.14	0.32	0.72
Maximum Voltage with 1 k $\Omega$ resistance (V)	0.14	0.19	0.36
Maximum Current (mA)	0.14	0.19	0.36
Maximum Power(mW)	18	36	144
Maximum Current density (mA.m <sup>-2</sup> )	0.57	7.92	16.7
Maximum power density (mW.m <sup>-2</sup> )	73.4	1440	7000
Internal Resistance (m $\Omega$ )	281	147	50
Coulombic efficiency (%)	0.6	0.8	1.8

The voltage generation is based on microbial electron transfer at electrode in case of membrane. Voltage was produced over time when starch was pumped into anode chamber.

Polarization curves are useful for determining dependence on resistance of fuel cell performance. To obtain the polarization curve, the current density was calculated and plotted against voltage and power density at different external resistances (1– 5 k $\Omega$ ).

Over the whole experimental period, the maximum power density of 73.2, 162 and 650 mW/m<sup>2</sup> occurred at 1 k $\Omega$  with a current density of 0.57, 0.84 and 1.76 mA/m<sup>2</sup> for 250 mgCOD/l, 525 mgCOD/l and 1050 mgCOD/l respectively.

When 1050 mgCOD/l starch is fed to the operated MFC system. Current decreased consistently with the increase in internal resistance, which is accordance with literatures reported earlier (Liu and Logan, 2004; You et al., 2006; Venkata-Mohan et al., 2008b).

It is evident from the experimental data that the voltage–current density curve can be roughly divided into three stages: activation polarization, ohmic loss and concentration polarization. At the first stage, current was relatively low, activation resistance caused by reaction kinetics played a dominant role, which caused a rapid voltage decrease. As current increased, polarization curve showed a linear relationship between voltage and current, which is called ohmic polarization, resulted from ionic resistance and electronic resistance. In this phase, there was also non-ohmic polarization; when external resistance was equal to internal resistance power density reached the maximum of 650 mW/m<sup>2</sup>. With continued increase of current density, concentration diffusion became obvious.

A maximum CE of 1.8% was obtained at external resistance of 1000  $\Omega$ , which is quite lower than CE of synthetic wastewater (Liu and Logan 2004). There are many possible reasons for such a low Coulombic efficiency.

The experimental studies performed in this Thesis showed that a large volume two compartment MFC could be used to generate electricity coupled with efficient COD removal. The designed and constructed MFC system equipped with relatively cheap electrodes (stainless steel plates) could be operated at similar current and power densities reported in the literature. Experiments with different feed concentrations showed that the MFC system could produce higher electricity outputs per unit

amount of organic matter at higher substrate concentrations with the same COD removal efficiencies. The studies presented in this work present the applicability of MFC concept into conventional suspended growth activated sludge systems. Thus, the study can be regarded as an initiation for upgrading the conventional activated sludge systems to generate electrical power using stable and inexpensive materials as electrodes, which will add to feasibility of MFC applications.

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